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AN ENTOMOPHTHORACEOUS TARDIGRADE PARASITE PRODUCING SMALL CONIDIA ON PROPULSIVE CELLS IN SPICATE HEADS

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Among the various microscopic animals that multiply in aging agar plate cultures prepared for the isolation of root-rotting fungi, and also in cultures which after being overgrown by a root-rotting oomycete have been further planted with decaying vegetable material, tardigrades, or bear animalcules, often making their appearance. The development of these animals, as has been intimated (Drechsler 1948), is unfortunate where the cultures are intended to be used in the study of fungi destructive to eelworms and rhizopods, for although their slow multiplication and leisurely movement makes them less violently ruinous than annelids and mites, they yet operate persistently like so many minute bulldozers to crush down and obliterate all of the more delicate aerial reproductive apparatus in their paths. They have seemed, in my experience, to be themselves rather little subject to fungus attack. During many years mycelia or other recognizable fungus bodies have been observed within bear animalcules only now and then. Recently, however, several Petri plate cultures revealed progressive destruction of a tardigrade that continued until all motile individuals were killed. The parasite concerned here was a remarkable member of the Entomophthoraceae that like the two related nematode parasites I have described under the binomials *Meristacrum asterospermum* (Drechsler 1940) and *Gonimochaete horridula* (Drechsler 1946) displays a distinctive primal manner of asexual reproduction by giving rise to small spores in comparison with which the familiar large conidia of the insectivorous forms may be regarded as morphologically equivalent to sporangia.

Both the fungus and its host animal were obtained from leaf mold gathered on February 14, 1950, along a roadside bordering a deciduous wood near Oxford, Pennsylvania. On adding small quantities of the forest detritus to maizemeal-agar plate cultures that previously had been inoculated and had become overgrown with mycelium of *Pythium irregulare* Buism., the tardigrade multiplied well for nearly a month to populate the 63 square centimeters of agar expanse in each of the several Petri dishes with three

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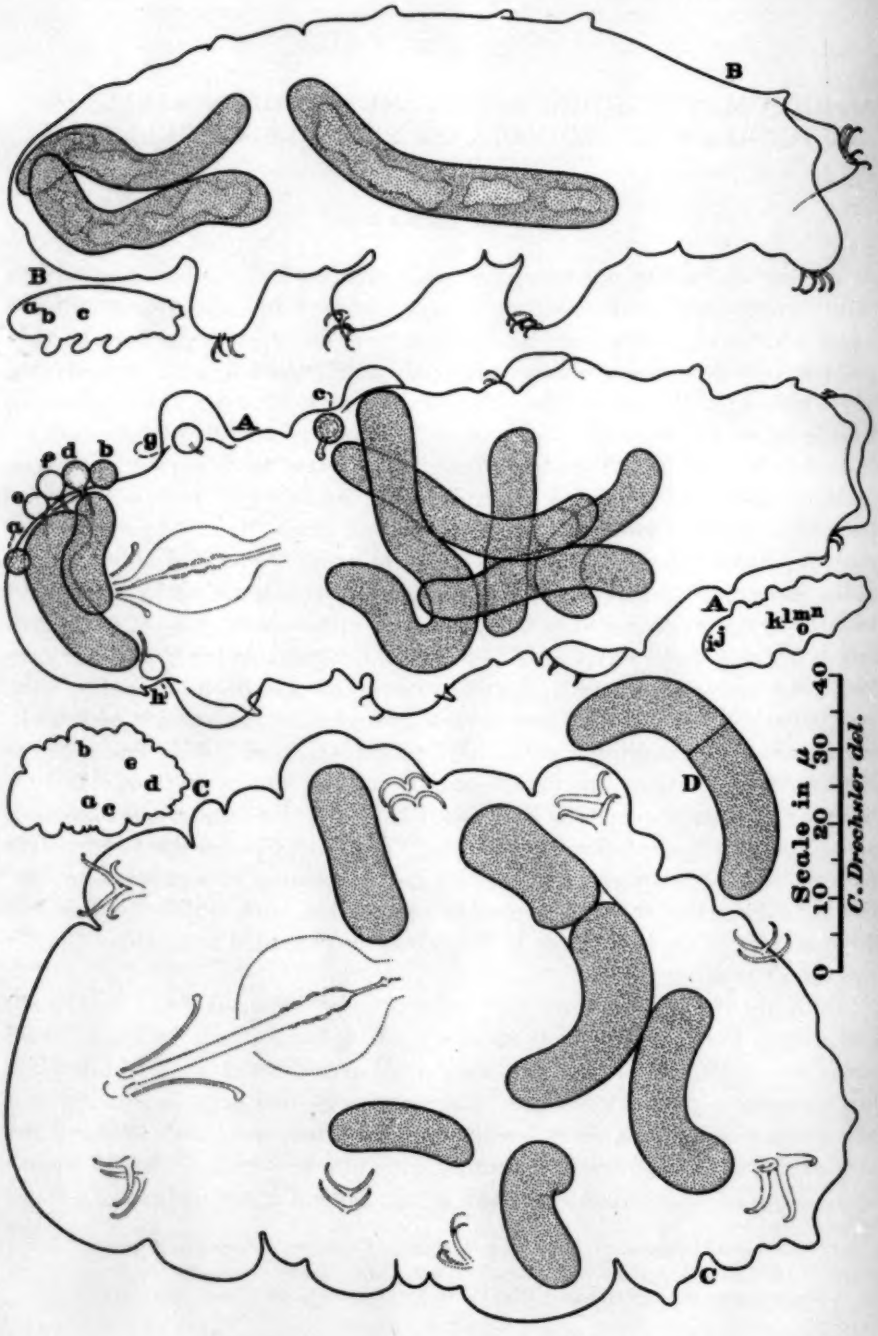
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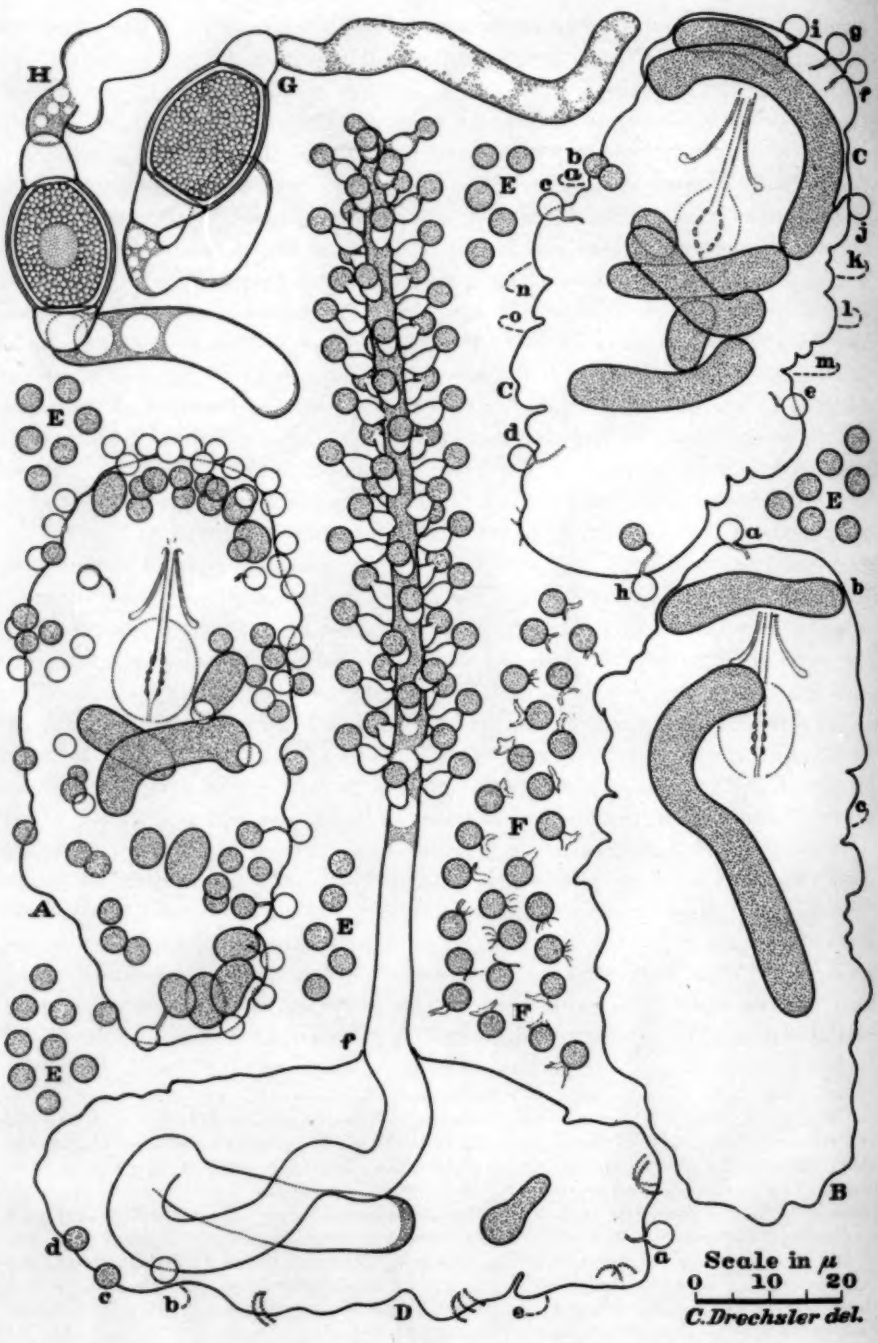
hundred to five hundred individuals of varying ages. Full grown animals measured about 200 μ in length and 65 μ in width. Cuticles of approximately these dimensions were found here and there, loosely enveloping clusters of five, six, or seven smooth eggs. Except for their legs and a considerable number of somewhat pointed warty projections on the dorsal side, the animals seemed lacking in noteworthy protuberances. According to the classification given in the fairly recent monograph by Marcus (1936) the species belongs clearly in the family Macrobiotidae of the order Eutardigrada. Since the two claws on each foot appeared generally of about equal size, it seems better referable to the genus *Macrobiotus* than to *Hysibius*, though I was not able to identify it with any one of the many species compiled under *Macrobiotus* by Marcus. Since the accompanying illustrations (figs. 1–5) are intended primarily to set forth the development of the parasite they can show little more than the outline of the animal's body as it appeared in each instance under the microscope. When the animal was already disabled by the fungus at the time it was mounted with the subjacent agar under a cover glass, its body outline, whether presented in dorsal (fig. 1, A) or in lateral view (fig. 1, B), kept approximately normal dimensions. Infected animals in whom development of the parasite was less advanced, often writhed beneath the cover glass, on exposure to upward illumination, for more than an hour in order to turn upside down, thereby bringing into view all four pairs of claws; and in the struggle they usually became flattened much beyond their normal width (fig. 1, C). As the animal's bare outline, especially in dorsal aspect, often showed too little difference between the front and rear profiles, the head end was marked in the figures, wherever space allowed, by indicating the position of the stylet and pharynx.

Infection of tardigrades always proceeds from externally adhering globose spores varying in diameter from 3 to 4.5 μ. In the region of contact between the individual spore and the animal's cuticle no deposit of adhesive secretion, nor any outward structural modification, is to be seen. While the spores seem capable of sticking to the cuticle about equally well in all parts, they usually beset the frontal region most abundantly, as, indeed, might be expected from the greater local frequency of encounter resulting from the forward locomotion of the animal (fig. 1, A; fig. 2, A; fig. 3, A). For some

FIG. 1. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Infected specimen of *Macrobiotus* sp. (dorsal view) showing two adhering conidia, a and b, that have not begun to germinate; two germinating conidia, c and d; three empty conidial envelopes, e-g; an empty conidial envelope with denuded germ tube, h; and seven assimilative hyphal cells, i-o. B, Infected specimen of *Macrobiotus* sp. (lateral view) showing three assimilative hyphal cells, a-c. C, Infected specimen of *Macrobiotus* sp. (ventral view, somewhat flattened) containing four unicellular assimilative hyphae, a-d, and a uniseptate hypha, e, which is nearly ready to separate into its component cells. D, Same hypha as shown in C, e, but drawn four hours earlier, soon after cross-wall was laid down.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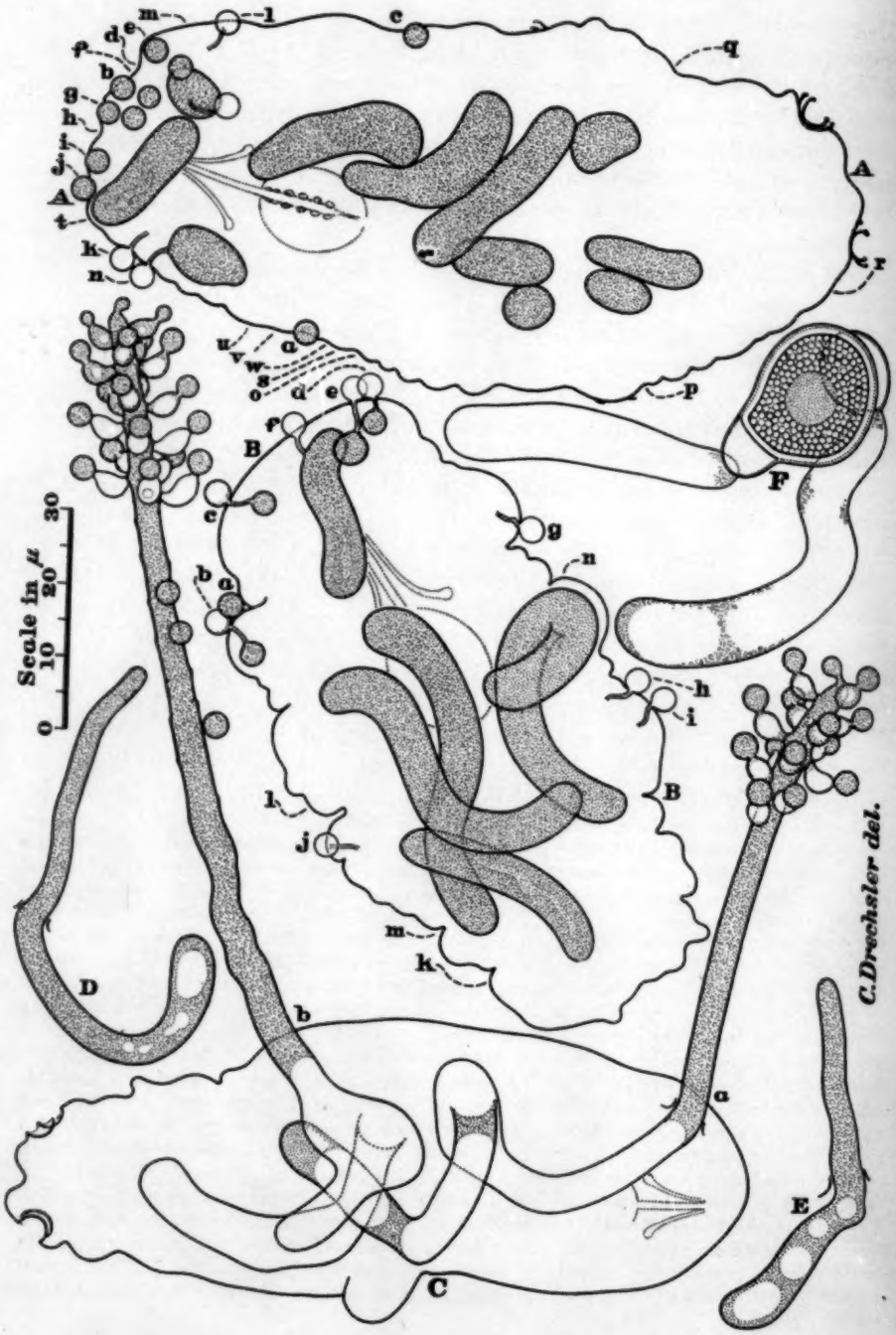
time after becoming attached the spore shows no germ tube, and remains filled throughout with protoplasm (fig. 1, A, a, b. fig. 2, A; C, a, b; D, c, d. fig. 3, A, a–j; B, a. fig. 4, A, a. fig. 5, B, a). Sooner or later a narrow germ tube burgeons forth from the adhering surface of the spore, perforates the animal's cuticle, and penetrates into the underlying fleshy tissue, while concomitantly a vacuole enlarges within the spore (fig. 1, A, c, d). Along the sides of the host animal as also at its rear and down its legs, the body wall is only a few microns thick, so that virtually all germ tubes intruded from spores affixed to the legs or adhering in lateral or posterior regions reach the body cavity before they cease elongating. Once its tip is in the body cavity each germ tube swells out terminally to form a globose infection bladder (fig. 3, B, b, c), into which are received, as a rule, the entire contents of the spore (fig. 2, A; C, h. fig. 3, B, d. fig. 5, B, b). The infection bladder now begins autonomous development as a thallus or assimilative hypha. In many instances it soon becomes abjointed through disturbances due mainly to the animal's locomotion, and gradually is floated away in the host haemolymph, leaving behind the empty spore envelope and slender germ tube (fig. 1, A, h. fig. 2, B, a; C, c–g; D, a, b. fig. 3, B, g–j. fig. 5, A) from which it originated. In the frontal region of the animal the infection bladder is rather often formed in the tissue under the cuticle (fig. 2, A; fig. 3, B, c, d), as the tissue here makes up a considerably thicker layer than elsewhere. Yet even in the fleshy head some empty germ tubes are to be found from which young assimilative hyphae have been abjointed (fig. 2, B, a; C, f, g. fig. 3, A, k, l). Often the germ tubes become indiscernible early, so that the connection between the empty spore envelope and the young assimilative hypha imbedded in the fleshy head, is no longer visible, though presumably still existent (fig.

FIG. 2. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Small specimen of *Marcrobiotus* sp. (dorsal view) to which are attached many living conidia as well as many conidial envelopes from which infection bladders have been intruded, though germ tubes are visible only in a few instances; within the animal are shown many small infection bladders and about a dozen somewhat larger hyphal cells. B, Small specimen of *Macrobiotus* sp. (dorsal view) showing an empty conidial envelope with denuded germ tube, a, and two hyphal cells, b and c. C, Small specimen of *Marcrobiotus* sp. (dorsal view) showing two adhering conidia, a and b; five empty conidial envelopes, c-g, each with a denuded germ tube; an affixed empty envelope, h, with a germ tube bearing an infective bladder at its tip; two empty affixed conidial envelopes, i and j, each having a germ tube connected with a sizable hyphal cell; and five separate assimilative hyphal cells of different sizes, k-o. D, Small specimen of *Macrobiotus* sp. showing an affixed empty conidial envelope, a, with denuded germ tube; an empty conidial envelope, b, whose germ tube is not visible; two adhering conidia, c and d; a small assimilative hyphal cell, e; and a large assimilative hypha, f, from which a conidiophore bearing about sixty conidiiferous branches has been extended. E, Random assortment of conidia as appearing on a moist substratum. F, Random assortment of conidia as appearing on rather dry agar soon after their discharge, each with tattered membrane of the lateral cell on whose tip it was produced. G, Unit of sexual apparatus with a nearly mature zygospore. H, Unit of sexual apparatus with a fully mature zygospore.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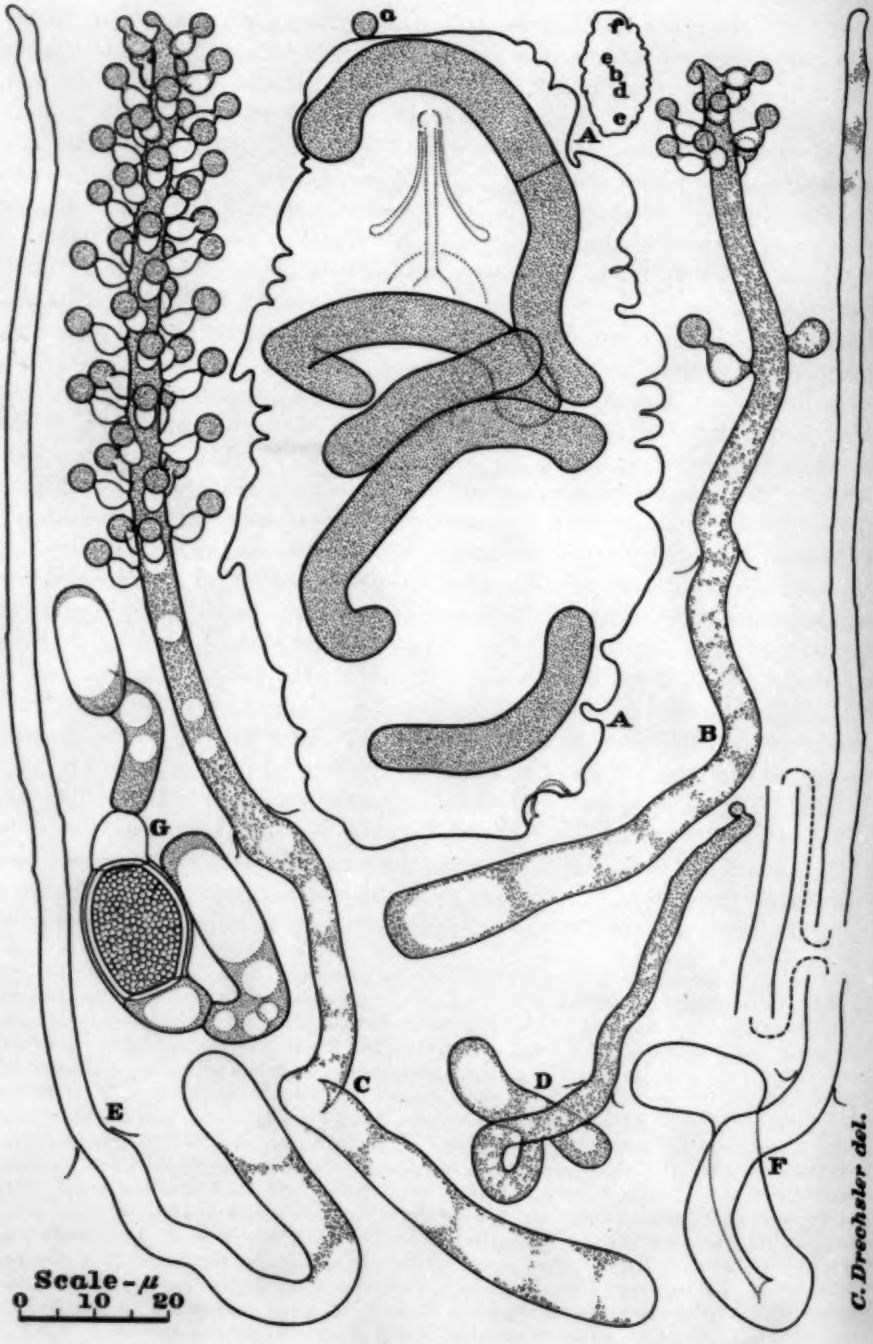
2, A). On the other hand, germ tubes not infrequently persist as visible connections at more advanced stages of growth, not only in the frontal region (fig. 2, C, i. fig. 3, A, m; B, e, f. fig. 5, A, a, b, g) but also in positions farther back (fig. 2, A; C, j. fig. 3, A, n. fig. 5, A, c–f). Where, in instances of such persistence, the thallus has grown into a short thick hypha, the germ tube is commonly found attached not centrally to one end of the hypha, but to the side of the hypha at some little distance from one end. In this respect the fungus shows noteworthy parallelism with *Meristacrum asterospermum.*

As has been intimated the thalli or assimilative hyphae achieve their early growth mainly in the haemolymph of the animal's body cavity. In this liquid ambient they are bustled around promiscuously until because of increasing size they become lodged between the organs of the host and its body wall. They thereby become settled in a pronouncedly haphazard arrangement (fig. 1, i–o; B, a–c; C, a–e. fig. 2, B, b, c; C, i–o. fig. 3, A, m–w; B, f; k–n. fig. 4, A, b–f. fig. 5, A, a–v; B, c–e), which, in conjunction with their frequently crooked elongation, makes for a disorderly appearance that contrast strongly with the neat orderliness usual in the vegetative hyphae of *Meristacrum asterospermum.* Unicellular assimilative hyphae of the tardigrade parasite are not often found exceeding 80 μ in length, for segmentation here is not delayed as in *M. asterospermum,* until vegetative growth has been virtually concluded, but may take place, especially where a large animal host has been infected from a single spore, during all except the earliest stages of growth. In some instances a hypha only about 40 μ long may show a median cross-wall (fig. 1, C, e; D). More often, however, median partitions are laid down in hyphae ranging from 50 to 75 μ in length (fig. 4, A, f). After a cross-wall has been formed the daughter segments become convexly rounded at the place of division (fig. 1, C, e), and then are easily broken apart by rather slight disturbance. When the dead or moribund host is no longer capable of furnishing any disturbance, disjunction would seem to ensue less freely, so that several segments may remain united and thus

FIG. 3. *Ballocephala sphaerospora,* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Small specimen of *Macrobiotus* sp. (dorsal view) showing ten conidia, a-j, adhering to its cuticle; two affixed empty conidial envelopes, k and l, each with a denuded germ tube; two empty conidial envelopes, m and n, each with a germ tube to which a growing assimilative hypha is attached; and nine detached assimilative hyphal cells of various sizes, o-w. B, Small specimen of *Macrobiotus* sp. (dorsal view) showing an adhering conidium, a; two nearly empty conidial envelopes, b and c, each with a germ tube terminating in an infection bladder; an affixed empty conidial envelope, d, with a germ tube terminating in infection bladder; two empty conidial envelopes, e and f, each connected by a germ tube to a growing assimilative hyphal cell; four affixed empty conidial envelopes, g-j, each with a denuded germ tube; and four separate assimilative hyphal cells, k-n. C, Small specimen of *Macrobiotus* sp. with two assimilative hyphal cells, a and b, each of which has given rise to a growing conidiophore; the conidiophore of b shows serrations where propulsive cells were attached earlier. D, E, Small assimilative hyphal cells, each of which has begun to put forth a conidiophore. F, Unit of sexual apparatus, with a fully mature zygospore.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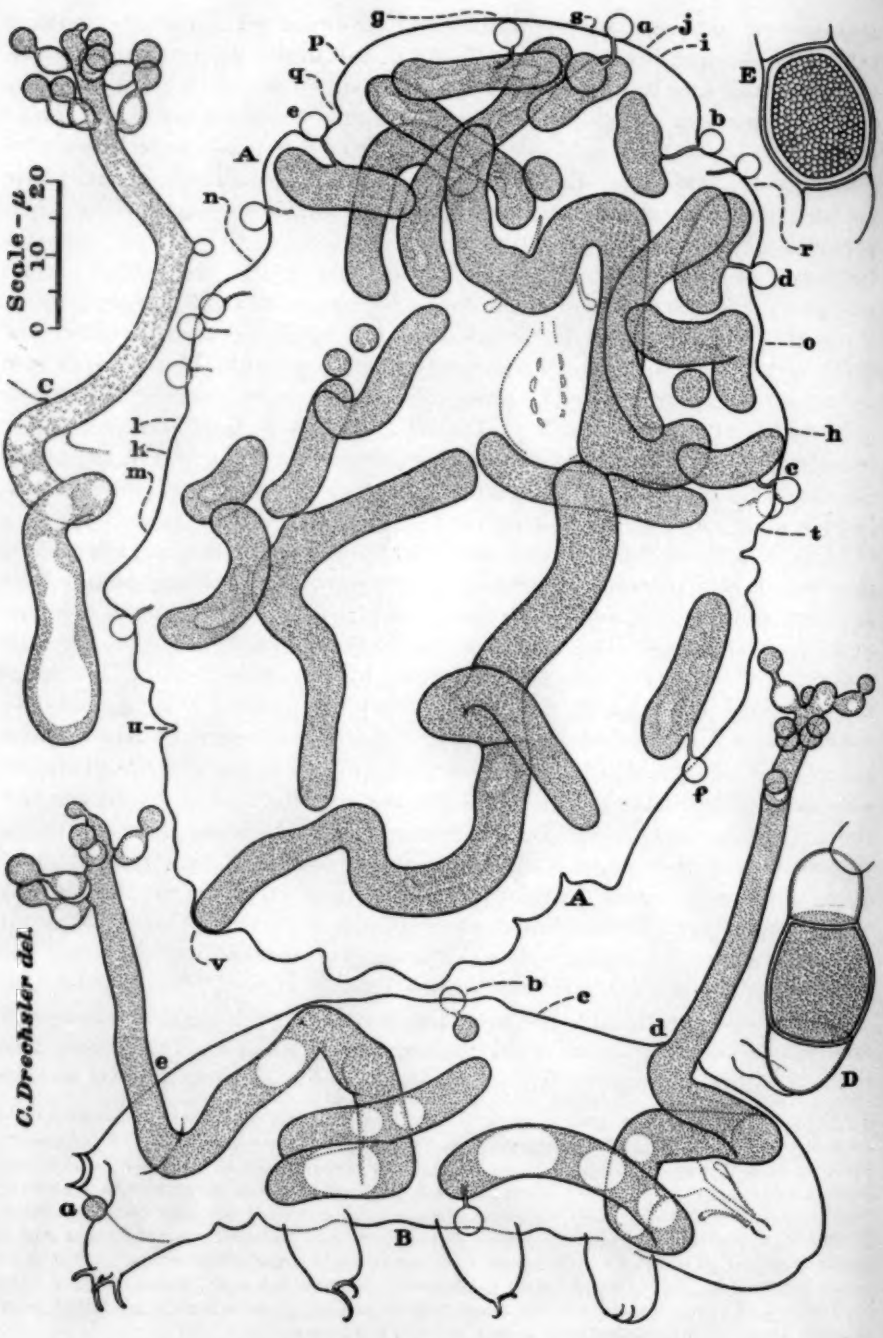
may appear as a sparsely septate, simple or branched filament extending crookedly through the animal (fig. 5, A, v). Naturally no hyphal segmentation occurs in host animals, which, as the result of being infected by many adhering spores at nearly the same time, harbor from the beginning a larger number of thalli than can develop to full size on the limited supply of nutrients available. The frequency of septation is manifestly governed by the size of the animal host as well as by the number of competing thalli present originally, since a small young tardigrade can provide nourishment for only 1 or 2 full-sized unicellular hyphae (fig. 2, D, e, f; fig. 3, C, a, b), whereas a large tardigrade is often found to contain 25 to 35 such bodies.

Asexual reproduction is initiated by the fungus some time after the death of the host, usually when a considerable portion of its substance still remains to be assimilated. The unicellular hypha or hyphal segment grows out broadly at one end (fig. 3, C, a; D; E. fig. 4, B, C. fig. 5, B, d, e; C), or less often puts forth a stout lateral branch (fig. 3, C, b; fig. 4, D). On reaching the host cuticle, the prolongation or branch forces its way through this barrier and continues growth upward into the air. At a height frequently of 25 to 50 μ, its apical growth is modified by the production at rather short intervals of conidiiferous branches outwardly much resembling the phialides familiar, for example, among the species of *Acrostalagmus*, *Cephalosporium*, and *Spicaria* parasitic on nematodes and insects. These branches are burgeoned forth one by one at the elongating bluntly rounded tip. In a moist microscopical mount under a cover glass they first become recognizable individually as a small globose or saccate protuberance connected narrowly with the hyphal tip (fig. 3, C, a, b; fig. 4, B, C). Very commonly the protuberance is found directed at nearly a right angle to the axis of the hypha. Although such posture might result from accidental bending incurred in the preparation of the mount it appears rather more likely that the protuberance is normally budded forth at an abrupt angle. In any event the growing protuberance very soon occupies an unquestionably lateral position as the hypha continues to elongate straightforwardly. On reaching definitive size it forms an ellipsoidal utriform branch usually curved in such wise that when viewed from the side the proximal portion dips noticeably downward, whereas the distal portion is directed markedly upward. The branch now becomes delimited basally through deposition of a cross-wall at its narrow

FIG. 4. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Specimen of *Macrobiotus* sp. (dorsal view) showing an adhering conidium, a; four unicellular assimilative hyphae, b-e; and a uniseptate assimilative hypha, f. C, Assimilative hyphal cell that has given rise to a growing conidiophore from which forty-six conidiiferous branches have already been put forth. D, Small assimilative hyphal cell that has grown out laterally to produce a young conidiophore. E, Denuded conidiophore and nearly empty assimilative hyphal cell from which it arose. F, Empty assimilative hyphal cell and denuded conidiophore arising from it; for lack of space, shown in parts whose proper connection is indicated by dotted lines. G, Unit of sexual apparatus with nearly mature zygospore.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attachment, while at about the same time it puts forth a globose bud distally. This globose bud gradually expands as it receives protoplasmic material from below. Eventually, when after increasing vacuolization the utriform part has yielded all its granular contents, the bud is walled off proximally as a full grown conidium. In the undisturbed humid atmosphere of a closed Petri plate culture the conidium may remain in place for many hours or, perhaps, for a few days, as the utriform cell, filled with clear liquid, retains its shape unchanged.

While the sequence of events in the production of the first lateral branch and of the conidium borne on it are taking place, the parent hypha continues its upward growth and at intervals of about 1.5 or 2 μ keeps on putting forth at its apex one utriform protuberance after another. Each successive protuberance then likewise undergoes development into a lateral branch and terminal conidium. Consequently when the first conidiiferous branch is completely formed, from 5 to 10 (as a rule, perhaps, 8 or 9) such branches have been put forth and have reached varying stages of development,—the lowermost one being nearly mature while the uppermost one is present in an incipient state. A progression of development corresponding approximately to that of an indeterminate inflorescence in phanerogamic plants is thus inaugurated. This progression is maintained as elongation of the conidiophorous hypha continues, until all the protoplasmic material elaborated below in the assimilative hypha or hyphal segment has been expended. Where the underlying assimilative unit is fairly large, from 40 to 80 conidia may eventually be produced (fig. 2, D, f; fig. 4, C). Transfer of protoplasmic materials upward is accompanied by increasing vacuolization below. It seems very probable, nevertheless, that the protoplasm available in the hyphal cell at the time the conidiophore begins to grow out is augmented considerably through continued expropriation of the host, for when reproductive development from all hyphae and hyphal segments is completed, little is left of the tardigrade except its cuticle and claws.

FIG. 5. *Ballocephala sphaerospora*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. A, Specimen of *Macrobiotus* sp. (dorsal view, somewhat flattened) showing seven affixed empty conidial envelopes, each with a denuded germ tube, and seven other such envelopes, a-g, each with a germ tube to which is attached a growing assimilative hypha; fourteen unicellular assimilative hyphal cells, h-u, of different sizes; and one branched biseptate assimilative hypha, v, whose lowermost segment seems ready to push a conidiophore through the host cuticle. B, Small specimen of *Macrobiotus* sp. (lateral view) showing an adhering conidium, a; empty conidial envelope, b, with a germ tube terminating in an infection bladder; a separate assimilative hyphal segment, c; an empty conidial envelope with a germ tube to which is attached a rather large assimilative hyphal cell that has put forth a young conidiophore, d; and a separate assimilative hyphal cell which likewise has put forth a young conidiophore, e. C, Another assimilative hyphal cell from which has arisen a conidiophore in rather early stage of development. D, Zygosporangium showing between the empty locules at its ends a young zygospore filled with finely granular protoplasm. E, Zygospore in nearly mature condition.

Although the axial hypha grows out about 1.5 to 2 μ before extending each successive protuberance from the apex, the resulting branch does not usually come off directly above its immediate predecessor, but is attached to a different sector, and thus in a plane parallel to the surface of the substratum makes a rather wide angle with its immediate predecessor. Generally the individual conidiiferous branches are separated by intervals of approximately 10 μ from their nearest fellows that project out in the same direction and that arise, above or below, from the same radial sector of the parent hypha. As the branches collectively occupy only an estimated one-third or one-fourth of the space available on the hypha, their arrangement is a loosely rather than a compactly spicate one. Viewed from above, that is from a position in alignment with the axial hypha, they show often some tendency toward pentastichy. Their rather even distribution and fairly regular spacing adds to the handsome appearance offered by a well-laden conidiophore in lateral view.

Since in a quiet humid atmosphere a full-developed conidiiferous branch may remain unchanged for a somewhat extended period, Petri plate cultures that have been kept closed for several days will contain here and there the cuticle of a dead host tardigrade from which perhaps more than twenty well-developed conidiophores and some smaller ones are found arising. Often nearly all of the more robust conidiophores erupt near the margin of the cuticle, and lean slightly away from it; so that together they make up, as it were, a palisade having a roughly elliptical form below and flaring out perceptibly above. The striking display thereby provided does not long endure when the cover of the Petri dish is removed. After a few minutes the handsome conidial apparatus will begin to disintegrate, and in less than half an hour may become desolate beyond recognition. Every conidiophore will then usually be found denuded not only of all mature conidia but also of the utriform lateral branches on which they were borne. Conidiophores that had completed their development will have been reduced to bare empty hyphae, with slight serrations marking the places where branches had been attached (fig. 4, E, F). Conidiophores that had been well advanced in their development but were still actively elongating, will have retained only the immature branches, usually numbering from 5 to 10, at different stages of production at the tip. Only rather young conidiophores without any mature conidiiferous branches will have remained unchanged. The agar substratum surrounding denuded conidiophores will have become thickly strewn with detached conidia. Mostly such scattered conidia appear as globose bodies with a clean circular profile (fig. 2, E). In some areas, however, where the agar would seem a little too dry at the surface for ready wetting of any small objects that may fall on it, the conidia show individually a film-like appendage, highly variable with respect to size and outline (fig. 2, F). This appendage evidently represents the tattered membrane of the lateral cell on

whose tip the conidium was produced. Since nothing with the shape and size and content of the lateral cells is ever observable among the scattered conidia, it seems certain that these cells undergo destruction when the spores are being shed.

The results achieved through their destruction may be inferred from the distribution of the conidia over the substratum. In instances where a single conidiophore had developed from a small tardigrade that succumbed in a rather isolated position, the detached spores will not be found limited to a small area around the base of the denuded hyphal shaft where they would have fallen under gravitation alone, but will be scattered over a considerably wider expanse. About four-fifths of the conidia will usually lie somewhat evenly distributed in a circular tract having a radius of 200 μ. By far most of the remaining spores will be sprinkled more sparsely in the adjacent concentric zone having its outer circumference 300 μ from the denuded hypha, while a few will be seen at distances between 300 and 400 μ. Evidently the conidia of the fungus do not merely fall from their attachments, but are forcibly thrown off. The propulsive energy is obviously supplied on rupture of the turgid envelope of the unicellular supporting branch. Unfortunately the bursting of the cell, as also the flight of the spore, is wholly indiscernible under the microscope. Judging from the membranous remnants attached to the discharged spores, the cell envelope probably ruptures at its basal end. It seems at least possible that momentum is imparted to the spore through recoil from violent backward squirting of sap. The mechanism operative here may therefore be of the "rocket" type whereby, according to Ingold (1934), the inflated basidium of *Basidiobolus ranarum* Eidam is projected into the air. As the distal portion of the supporting cell is directed noticeably upward, there is reason to presume that the conidium is shot off ascendingly, and thus has a longer trajectory than if it were shot off horizontally.

Shortly after all their mature lateral cells have been discharged, conidiophores—especially conidiophores closely surrounded by many others—are often found with individual spores adhering to them, or with chains of 2 or 3 or 4 spores drooping from their tips. Many such adhering spores unquestionably come from neighboring conidiophores on normal discharge of the lateral cells that produced them. Lodgment of spores on their own conidiophores can perhaps take place only through mishap or faulty operation of the discharge mechanism. The generous spacing of the lateral cells on the hyphal shaft strongly recalls the uniform separation of basidia noted by Buller (1909) in the hymenium of species of *Coprinus*. If, as Buller suggests with reason, the wide spacing of basidia serves helpfully in preventing the sticky basidiospores from touching one another during development and discharge, the separation of the lateral cells in the present fungus can well be interpreted as an adaptational feature of like utility. Of course, strong adhesiveness of

the conidia is here a character of very obvious necessity, for through it alone can the fungus get and keep a foothold on the cuticle of the host animal preliminary to infection.

When the one or two conidiophores usually found produced from small young tardigrades have discharged their conidia, the substratum over an area of about .2 square millimeter is bestrewn with spores so thickly that any visiting host animal would seem to have little prospect of not becoming infected. Where a fully grown tardigrade gives rise to a score of conidiophores arranged in a flaring palisade, the ensuing discharge provides a similarly effective cover over an area of approximately .6 square millimeter. These areas are, indeed, small in comparison with the macroscopically visible fields of heavy conidial contamination that often surround insects killed by entomophthoraceous parasites of the robust type long familiar. Pronounced disparity in range of projection is not surprising in view of the wide difference in size of both the spores and the associated propellent parts. From assigned linear dimensions it may be computed that the conidia of the tardigrade parasite are exceeded in volume fully two hundred times by those of the house-fly fungus, *Entomophthora* (*Empusa*) *muscae* (Cohn) Nowak. and from one thousand to two thousand times by those of *Entomophthora* (*Empusa*) *papillata* Thaxter (1888). The disparity in range of projection seems expressive of adaptation to wide differences with respect to unobstructed space available under natural conditions. Infected insects usually succumb in exposed positions, where ordinarily there is ample scope for long trajectories, while terricolous tardigrades of the kind here concerned live and die mainly in the narrow interstices within accumulations of decaying vegetable materials.

In agar plate cultures of customary firmness the tardigrades move about and succumb to infection mainly on the surface. Therefore, when the prolongations from the hyphal segments break through the host cuticle at the front or rear, along the sides, or from the back, they usually emerge directly into the air and so are favorably situated to begin forming conidia after only little additional growth. Yet in limited areas where the agar has become softened under small deposits of water, infected tardigrades occasionally bog down and thus eventually succumb in submerged positions. The hyphal prolongations emerging from such animal hosts first push their way through the overlying ambient before they begin to produce conidia. As in *Meristocrum asterospermum*, conidia here are formed only on aerial terminal portions of the outgrowing hyphae. Although under natural conditions the host animal with its weak legs and broad frontal conformation seems unlikely to bore into the individual firm pieces that make up plant detritus, infected specimens undoubtedly become submerged now and then through the ordinary accidents of water movement. A noteworthy postural feature of the

fungous comes to light when infected animals succumb on the vertical faces of an agar block that has been placed on the flat expanse of a Petri plate culture. The conidiophores, in these instances, grow out more or less horizontally, that is, in a general direction normal to the vertical surface concerned.

The fungus shows distinctive departures from the developmental trends displayed by *Gonimochaeta horridula* and *Meristacrum asterospermum*. In *G. horridula* all segmentation takes place rather soon after the host has been infected, the products of repeated fission there being only a little larger than the infective bodies intruded from the adhering conidia, and consequently much smaller than the thallodic cells that eventually will put forth reproductive outgrowths. In *M. asterospermum*, on the other hand, hyphal growth proceeds without segmentation until virtually all the digestible substance of the host has been assimilated; the hyphae thereupon dividing by fission until the resulting segments have the reduced length at which asexual or sexual reproduction is habitually initiated. With respect to vegetative division the tardigrade parasite reveals a condition intermediate between the extremes represented in the two related forms; for, as has been noted, fission here occurs concomitantly with assimilation and growth, so that the hyphal cells never come to exceed the dimensions suitable for reproductive development.

However, in the organization of its conidial apparatus the tardigrade parasite is not intermediate between the two related small-spored fungi, but clearly shows an advance beyond *Meristacrum asterospermum*, just as that fungus shows an advance beyond *Gonimochaete horridula*. In *G. horridula* the aerial hyphal outgrowths into which the protoplasmic contents of the thallodic segments migrate, reveal a sequence of development like that of a determinate inflorescence. Spore formation there is always delayed until the outgrowth has reached its definitive length. The endogenous spores are first individualized at the tip of the outgrowth, and later in successively more proximal portions. They are also discharged in a more or less basipetal sequence, all owing their rather feeble propulsion to the pressure exerted by the clear liquid filling the thallodic segment below. In *M. asterospermum* development of the sporophorous outgrowth again proceeds as in a determinate inflorescence, but here the outgrowth, instead of forming spores endogenously, becomes partitioned at short intervals, and each of the resulting segments burgeons forth a conidium laterally. The condium evidently is shot away somewhat feebly through bursting of the parent segment, whose wall thereby collapses utterly. Apparently as a device to keep conidia from sticking to one another the fertile distal portion of the sporophorous hypha is convolved in a corkscrew-like spiral, and the conidia arise along the outer curvature of the helix. Development of the conidiophore in the tardigrade

parasite is distinctive in that it proceeds after the manner of an indeterminate inflorescence. The aerial shaft here does not become partitioned transversely but instead puts forth many phialide-like lateral cells, each of which produces and later shoots off a distal conidium. With respect to arrangement of axial hypha and conidiiferous cells the tardigrade parasite invites comparison, among nematode-killing hyphomycetes, with various species of *Acrostalagmus,* whereas *M. asterospermum* more nearly resembles my *Meria coniospora* (Drechsler 1941). Despite their different cellular relationships, the conidia of the tardigrade parasite seem strictly equivalent to those of *M. asterospermum,* which from similarities in size, shape, and plural development, appear homologous with the small secondary spores that are produced plurally from the large primary conidia of *Delacroixia coronata* (Costantin 1897) Saccardo & Sydow 1899. These secondary spores, or "microconidia" as Kevorkian (1937) termed them, seem interpretable on grounds set forth earlier (Drechsler 1946) as being equivalent to the zoospores of the Oomycetes, and the sporongiospores of the Mucorales. In accordance with such interpretation the large conidia of *D. coronata,* and by extension more especially also the large multinucleate conidia borne on unbranched conidiophores of the insectivorous forms commonly referred to *Empusa,* would correspond in a morphological sense to the sporangia of the Oomycetes and Mucorales. The conidia of the tardigrade parasite thus appear to be of a more primitive type than the large derivative conidia familiar among the Entomophthoraceae.

The fungus was found producing zygospores rather sparingly. In most infected animals no zygospores were formed at all. Usually only one or two developed within an individual tardigrade host, though in a few animals as many as seven were observed. Among the many intertangled thallodic segments often present, units of sexual apparatus are in large part commonly obscured from view. Sometimes the parent segments become largely indiscernible even while the zygospore is still in a young condition (fig. 5, D); or frequently again, a zygosporangium containing a nearly mature zygospore will be indistinguishable at either end (fig. 5, E). Where a reproductive unit in a nearly mature (fig. 2, G; fig. 4, G) or fully mature (fig. 2, H; fig. 3, F) state is clearly visible throughout, it reveals a hyphal segment at each of the two ends of an elongated ellipsoidal zygosporangium. The two hyphal segments, which sometimes are virtually empty of living material, and at other times retain a substantial quantity of protoplasm, evidently represent the parent cells from which were delimited the gametangia whose fusion resulted in the zygosporangium. At each end the ripening zygosporangium is usually empty for a distance equal to about one-fifth of its length, the empty portions appearing as dome-shaped locules. Occasionally a locule may keep a considerable remnant of protoplasm (fig. 4, G). Between the two

locules is contained the zygospore proper. Of prolate ellipsoidal, or dolioform shape, it occupies the full width of the zygosporangium, usually, indeed, causing that structure to bulge out noticeably. Except at the ends its thickish brown wall is adnate to the thin colorless zygosporangial envelope. At maturity the zygospore has a distinctly brownish coloration. In its interior a parietal layer of coarsely granular texture surrounds a homogeneous reserve globule.

To signalize a characteristic feature of its conidial apparatus the fungus is described under a generic name compounded of two words meaning "to throw" and "head", respectively.

Ballocephala Drechsler, gen. nov. Hyphae assumentes intra animalia viva crescentes, simplices vel aliquanto ramosae, interdum aliquid septatae, denique cellulis eorum saepe disjungentibus; cellula assumenti aut hypham fertilem aut sporam perdurantem (zygosporam vel azygosporam) gignente; hypha fertili in aerem se porrigente, in apice ramum lateralem pullulascente, deinde identidem recrescente multos alios ramos laterales deinceps proferente; ramo laterali in una cellula constante, in apice unum conidium ferente et postea id violenter propellente.

Assimilative hyphae growing parasitically within living animals, simple or somewhat branched, sometimes rather sparingly septate, the cells later often separating from one another; the assimilative cells each eventually putting forth a conidiophore or participating in the development of a zygospore or of an azygospore; the conidiophore after growing some distance into the air giving rise at its tip to a lateral branch, and then on repeated elongation burgeoning forth many other such branches, one by one, in successively more distal positions; each lateral branch made up of a single cell, and forming distally a single conidium which subsequently it propels forcibly into the air.

Ballocephala sphaerospora Drechsler, sp. nov. Hyphis assumentibus incoloratis, continuis vel paululum septatis, in singulis vel aliquot cellulis 5–80 μ longis et 5–10 μ crassis consistentibus, cellulis vulgo facile secedentibus; hyphis fertilibus incoloratis, simplicibus, plerumque 50-175 μ longis, basi saepius 4–5 μ crassis, sursum leniter attenuatis, apice circa 3 μ crassis, 20–80 ramulos conidiferos in laxum spicum ferentibus; ramulis conidiferis utriformibus, aliquid curvatis, 4–7 μ longis, 3–4.5 μ latis; conidiis laevibus, incoloratis, globosis, 3–4.5 μ in diametro, tubulum germinationis 2–5 μ longum et circa .5 μ crassum in tardigradum intrudentibus; zygosporangio inter duas cellulas assumentes oriundo, elongato-ellipsoideo, plerumque 28–33 μ longo, 14–16.5 μ lato, membrana ejus incolorata, .3–.4 μ crassa; zygospora elongato-ellipsoidea vel dolioformi, 17–20 μ longa, 13.5–16 μ lata, membrana ejus brunnea, .6–.8 μ crassa, cum membrana zygosporangii late concreta.

Speciem Macrobioti enecans habitat in humo silvestri prope Oxford, Pennsylvania.

Assimilative hyphae colorless, most often consisting of a single cell 5 to 80 μ long and 5 to 10 μ wide, but sometimes found composed of 2 or 3 or 4 such cells; the growing cells, whether separate or united, commonly dividing by means of a cross-wall on reaching a length between 40 and 80 μ, with the resulting segments usually soon becoming disjointed. Conidiophores

colorless, unbranched, mostly 50 to 175 μ long, 4 to 5 μ wide at the base, gradually tapering upward, near the tip about 3 μ wide, usually bearing 20 to 80 propulsive conidiiferous branches in open spicate arrangement; the propulsive branches unicellular, of utriform shape, mostly 4 to 7 μ long and 3 to 4.5 μ wide, usually curved with the distal portion directed upward; conidia colorless, globose, smooth, 3 to 4.5 μ in diameter, on adhering to a tardigrade host intruding an infective body by means of a germ tube usually 2 to 5 μ long and about .5 μ wide. Zygosporangium produced between two assimilative hyphal cells, of elongated ellipsoidal shape, measuring commonly 28 to 33 μ in length and 14 to 16.5 μ in greatest width, having a colorless wall, .3 to .4 μ thick, which is extensively adnate to the zygospore wall; zygospore elongated ellipsoidal or dolioform, often 17 to 20 μ long and 13.5 to 16 μ in greatest width, its proper wall distinctly brownish, and .6 to .8 μ thick, its protoplast at maturity consisting of a coarsely granular parietal layer and a central reserve globule 4 to 5 μ in diameter.

Parasitic on *Macrobiotus* sp. it occurs in deciduous leaf mold near Oxford, Pennsylvania.

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A FUNGUS CAPABLE OF PARASITIZING WHARF-PILING BORERS

B. O. DODGE

The average layman is apt to look upon the fungi as agents of disease and destruction rather than as organisms on the whole highly beneficial. On the other hand, the economic entomologist is always interested in the discovery of fungi that parasitize insects. Many such fungi are now well known. During the past summer my attention was called to the damage being done to certain New York wharf piling. While examining specimens for possible wood-rot fungi it was discovered that some of the borers causing much of the damage were being killed by a white mold. Just how important this fungus may prove to be, even under the most favorable conditions for its growth, is a question. The fungus itself is, nevertheless, of much interest because it turns out to be closely related to certain very important entomogenous fungous parasites.

On July 10, 1950, Dr. William J. Robbins, Director of The New York Botanical Garden, wrote me as follows:

"The wood I sent you comes from piles at Whitehall Street Ferry Terminal. The decayed wood was obtained around the limit of average water level. They (the pieces) were brought to us by Mr. H. N. Blume,[1] Department of Marine and Aviation, New York City, whose office is 39th Street Ferry Terminal. The insect causing the damage was identified at the Zoo. Mr. Blume would be interested in knowing anything about the fungi."

The wood samples were more or less decayed and marked by numerous burrows, but there was enough discolored rather solid wood to test for a possible wood-rot fungus. Transplants of freshly exposed pieces were made to agar plates. As a check Dr. P. P. Pirone made a similar planting of several pieces that had been surface sterilized with diluted Clorox. Except for a few obvious contaminants, the same beautiful snow-white growth finally developed from most of the transplants. Some of the cultures showed in the reverse side a pale rosy or purplish chromogenesis.

[1] Mr. Blume, who is engineer in charge of the Marine Borer Investigations of the New York Harbor, and Dr. Ross F. Nigrelli, New York Aquarium, consultant biologist for the Department of Marine and Aviation, have furnished certain information desired on the stages in the development of these borers, and on the piling-borer situation in general. They are referring to these insects as "Dutch Wharf Borers, *Nacerda melanura.*" The identification will, no doubt, be further checked by entomologists specializing in beetles of this group. The phrase "limit of average water level" would be expressed technically: "Level of Mean High Water Datum."

Here and there on the well rotted wood fragments, dry powdery snow-white masses composed of myriads of minute spores, which were very difficult to wet, were present. The spores were about 1–1.5 μ in diameter, and so might readily be carried into the wood along with capillary water. The mycelium which developed from the surface-sterilized pieces of wood may have come from such spores.

Some time later, we received additional specimens of damaged top-ends of piling (yellow pine?). On splitting the end blocks I found a number of larvae, about an inch or so long, actively at work as borers. The same white mold was present in spots on the decayed wood. A later sample showed, not only lively borers, but also a few that were evidently sick. A few of each kind were put in tubes of nutrient agar. The healthy ones lived on for several days burrowing around in the agar which had now usually developed contaminating fungi such as *Mucor, Aspergillus, Trichoderma,* etc., brought in with the larvae. The "sick" larvae soon died and developed remarkably pure cultures of the white mold.

As days passed and additional material was sent to me, it was apparent that we were dealing with some fungus (or fungi) that could occasionally attack the borers. Masses of snow-white growth composed mostly of spores lined the burrows for about the length of the dead borers (fig. 1. a, b).

The last sample was received about November 13th. Only three active borers were recovered. One of them was put in a sterile plate of potato dextrose agar and inoculated with powdery spores from an old plate culture by dusting them on the larva as well as on the nutrient medium. Another larva was placed in the old plate culture from which the spores were taken to inoculate the first borer. In the meantime, cultures and some mummified larvae were given to Dr. Rhoda Benham of the Department of Dermatology, P. and S., Columbia University. She is much interested in the fungi that parasitize animals, and has volunteered to make a study of the cultural features as well as the methods of spore formation. She is also studying sections of infected larvae that had died. She pointed out to me that much of the mycelia and spore-like bodies inside the borers appears to be coarser than are the hyphae and spores that make up the felt-like more superficial growth on the larvae sectioned. Dr. Benham will cover this feature in a future communication.

The two apparently healthy larvae that had been powdered with spores, as noted above, both developed a dense coating of mold (fig. 1. c, d), the mycelium of which also penetrated through the body of the borer. Transfers of bits of the mycelium from the dead borers shown in the figures, developed seemingly pure cultures of the same fungus.

On November 8th, reporting to Mr. Blume's office, I wrote in part: "It could be that this fungus may be of some help in controlling the borers." It

is well known that fungous parasites of grubs of the June beetle and of the adults of the common house fly, for example, may, under favorable conditions, keep down the population of these insects, but we still have some years a lot of June beetles and house flies. I mentioned to Mr. Blume that the fungus might in some way soften the wood and so render it more liable to attack by borers. Tests, however, should be made by inoculating sterilized

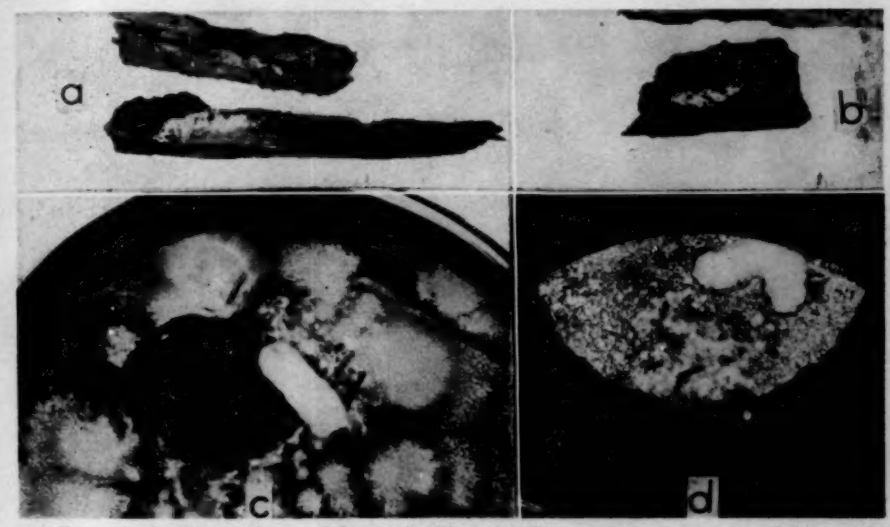

FIG. 1, a, b. Three pieces of decayed piling showing masses of spores and fragments of hyphae lining the burrows of certain piling borers that had died and had been removed for study. Cultures made from the spore masses and hyphal fragments develop the snow-white fungus even after having dried in the laboratory for several months. These particular cultures do not seem to show the rosy chromatogenesis;

FIG. 1, c. A portion of an old plate culture in which the living larvae had been placed earlier. A piece of the somewhat dried plate agar was later cut out to provide a "well" to hold some sterile water to keep the fungus in the borer from drying up. If allowed to dry too much it could not very well complete its life history. The hoped-for results were not forthcoming;

FIG. 1, d. A piece of the plate culture showing many small colonies of the white fungus that developed after the spores had been dusted on the sterile plate, thereby powdering the larva at the same time, as noted in the text. Dr. Benham stained sections of this larva. They show that the mold hyphae penetrated throughout the borer body. Cultures from bits of mycelium from the surface mold show the rosy chromogenesis. So we have here evidence that the fungus was parasitic.

pine wood with the fungi in piles attacked by these borers. Furthermore, we want to know whether or not the chromogenic and non-chromogenic isolates of this snow-white mold, noted above, represent different strains of the same species or do they represent two distinct species. The chromogenic factor may sometimes express differences in color on the media used at the time. We have, nevertheless, grown both chromogenic and non-chromogenic strains on opposite sides of the same plate and found that the color character

remains a clear mark of distinction. (See below for more on this point.)

I originally suspected that this white fungus might be an *Actinomyces*, because of the character of the mycelium and spores which could not be wet easily. On old plate cultures droplets of water of guttation covered with dry spores roll around on the plate like drops of mercury on a table. It was soon proved by culturing the fungus that it could not be an *Actinomyces*. Its occasional association with dead or dying larvae then suggested that it might be the imperfect stage of some member of the order Hypocreales and perhaps in the Cordyceps group. In any case the perfect or sexual stage would be essential for a correct identification.

Some years ago certain of our lawns at The New York Botanical Garden were badly infested with the hairy chinch bug, *Blissus hirtus*. The following year we found that the bug population was being rapidly reduced by a "milk-white" fungus. Normal bright reddish or orange-colored young soon after being infected turned a dull olive-gray color and died. Dead adults were usually covered with masses of very small white spores. Pure cultures of the fungus were easily obtained. It was noticed that a distinct reddish coloration usually extended out into the medium beyond the colony growth. Healthy young bugs that were artificially infected died.

Since chinch bugs had been reported subject to attack by *Sporotrichum globuliferum* Spegazzini, and as our fungus seemed to correspond morphologically with that species this name was used at the time (See Dodge & Rickett, Diseases and Pests of Ornamental Plants p. 115. 1948). Spegazzini first found this species on dead carabid beetles. Pettit (Cornell Agr. Exp. Stat. Bull. 97, 1895) reported extensive experiments with a number of entomogemous fungi, among them was *S. globuliferum* isolated from a carabid beetle. He also studied in culture the strain used by Forbes of Illinois in his work on this chinch-bug parasite. Both Forbes and Pettit observed the purplish color in their cultures. This *Sporotrichum* has been placed in the genus *Beauveria* by later authors. Beauverie himself (Rev. Gen. Bot. vol. 26, 1914) discusses rather critically various related species which he shows should be included in *Beauveria*. He describes in some detail four species, all of which are parasites of the silk worm. The disease "muscardine", has been the subject of much study in France. "La muscardine ordinaire" is caused by *B. Bassiana* (Balsamo) Vuill., while "La muscardine rouge" is caused by *B. effusa* Beauverie. Morphologically these two fungi are much alike, but Beauverie makes much of the rosy or reddish color imparted to the mummies and to cultures on certain media by the latter species. Were it not for his spore measurements, 2–4 μ in diameter, we would suspect that our non-chromogenic and chromogenic forms might be the two species he seeks to separate not only on the basis of chromogenesis, but also on the gross growth characters shown in culture. We have no difficulty in distinguishing our

two forms as cultures age. Beauverie, figures 9 and 10, shows characteristic differences in growth types. Spore measurements of our races made for us by Dr. Donald Rogers indicated that there is considerable variation in the size of the conidia: 1–2½ μ. Spegazzini and Beauverie, as well as Pettit, found the conidia to be 1.5–2.5 μ for *Sporotrichum globuliferum*.

Since the genus *Beauveria* as now understood includes species that were originally placed in *Botrytis, Isaria, Sporotrichum, Spicaria* and others, we shall not go far astray if we place both our non-chromogenic and chromogenic races in *Beauveria,* the chromogenic race being nearest to *B. effusa* as *Beauverie* describes that species. It could, however, judging by its spore measurements be identified as *B. globulifera*.

The general situation here calls for much further work on questions of identification from a purely taxonomic standpoint as well as from the host-parasite relationships involving these piling borers and their occasionally associated fungous parasites. So far as those with whom I have communicated know, no one has reported such parasites of the larvae of the beetle *Nacerda melanura*.

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THE PERISTOME OF CLASMATODON PARVULUS (HAMPE) SULL.

ELVA LAWTON

During the summer of 1950, the structure of the peristome of *Clasmatodon parvulus* (Hampe) Sull., was brought to the attention of the moss clinic at the Lakeside Laboratory of the University of Iowa, when Dr. Eula Whitehouse found that the peristome of her specimens from Texas did not conform to the description given in Grout's "Moss Flora of North America."

Grout (1934) states that the peristome is single and composed of sixteen irregular, filiform teeth. By his description, Brotherus (1925) considers it an outer peristome, when he says, "inneres Peristom fehlend." Since the collections from Texas showed very clearly the double nature of the peristome, further study was undertaken of recent collections, of the herbarium specimens of the early collections, and of the literature.

Hampe who described the moss first as *Leskea parvula* in 1839, gives no information about the peristome. But Wilson who named it *Clasmatodon* in 1842, describes specimens collected by Drummond near New Orleans, and says that the peristome is double, with sixteen short outer teeth, divided into two parts, and an inner divided membrane of just as many bifid or double parts placed somewhat irregularly opposite the outer teeth.

In 1851 C. Müller described a double peristome for *Clasmatodon* from southern United States. However, in 1852, Bruch, Schimper and Gümpel disagreed with Müller, for they state that "the peristome is simple and composed of sixteen teeth which show all the characteristics of an outer peristome not only by their origin, but by their structure and we cannot understand how the author of Syn. Musc. Frond. can consider them an inner peristome. The inner peristome does not really exist in this genus except in a rudimentary state, and is only indicated in the form of a pale irregular torn membrane, and cannot truly be considered as an inner peristome."

One cannot but wonder whether Müller ever really saw the outer peristome, for in his detailed description and discussion of the genus in 1875, he agrees with Schimper, as indicated by the following translation of a part of his article; "in the first place, the structure of *Clasmatodon* has not been well understood. As a rule it is obtained from North America with unripe or poorly developed fruits, and one is pleased to get it at all; there is little chance to make a thorough examination. However, I was very for-

tunate that J. Boll sent a specimen in all stages of development which he collected in northern Texas. I have investigated a number of the ripe capsules, and as a result of these observations, at last the true nature of the peristome of *Clasmatodon* is clarified. In general each capsule shows a different structure of the mouth and this explains clearly why each observer and each illustration seemed to give a different peristome structure. To put it briefly, it is an outer peristome and consists of sixteen lanceolate teeth. On the whole these teeth never reach the full development expected; the substance along the midline is insufficiently developed and here the tooth splits either into two parts of equal length, or one short and one long, and sometimes one tooth splits into three parts. Often the separation extends to the base of the mother tooth, but sometimes the base remains whole and shows a tooth with horizontal lines."

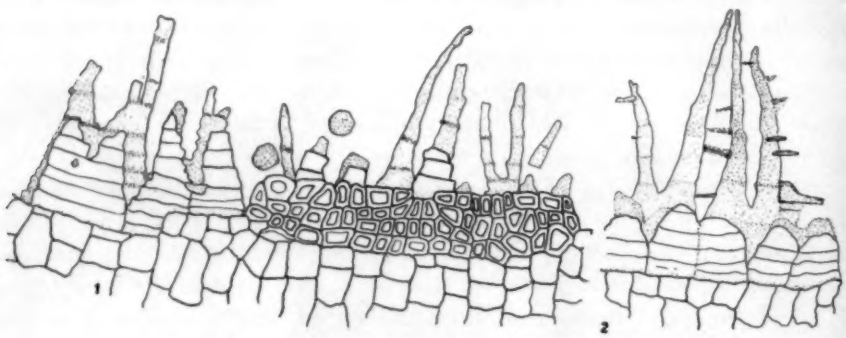
In the meantime, Sullivant in 1864 described and illustrated the peristome of *Clasmatodon,* and although he states that it is single, he goes on to say that on the dorsal side of the teeth at the base, there are sometimes one or two very short scale-like processes. These processes are clearly illustrated in his plate and they have the horizontal lines of a typical outer peristome, and in his figure 17, he shows the outer peristome teeth or processes fused with the inner. Furthermore, his figure 18 of a longitudinal section of the peristome brings out the true nature of the scale-like processes, since by position they conform to outer teeth.

The disagreement as to the structure arose, at least in part, because *Clasmatodon* and *Habrodon* were sometimes confused. Limpricht (1895) points out that the drawing of the annulus and peristome of *Rhegmatodon parvulus* (*Clasmatodon*) given by Hampe in 1844, is not of *Clasmatodon,* but of *Habrodon.* Schimper in 1860 states that Müller confused the two genera, and Braithwaite (1902) shows that both Müller and Schimper confused them; Schimper in Bryologia Europaea united them under the new name, *Anisodon perpusillus.* Hampe's figure shows a large annulus of the type found in *Habrodon,* but not in *Clasmatodon,* and a single peristome with the horizontal markings of an outer peristome.

The difficulties which Müller described in 1875 in regard to obtaining well developed material, may account for the fact that the outer teeth have not always been observed. Many specimens have been collected after the operculum was shed, and they show only broken teeth or none at all on many capsules; the few capsules with operculum intact are often smaller and depauperate, with the outer peristome so poorly developed that it scarcely extends above the annulus and is therefore overlooked.

Numerous collections from Texas, Georgia and Florida were examined, including a specimen in the herbarium of the New York Botanical Garden marked as collected by J. Boll in northern Texas, but without a date. There

is no doubt but that the peristome is double, with the outer teeth much shorter than the inner. Dr. Domke tells me that the herbarium at Hamburg has a specimen of *Clasmatodon* from Müller's herbarium which was collected by Boll in northern Texas in 1869, and it seems very probable that this specimen and the one in the New York Botanical Garden are a part of the material which was described by Müller in 1875 as having a single peristome. Specimens of no. 93 of Drummond, with the description of Wilson printed and attached, stating that the peristome is double, are in the herbarium of the New York Botanical Garden; however, no mount was made of this material, or of capsules from Sullivant's no. 246, because, from their descriptions, it seemed to be clear that both authors saw the outer peristome. The structure of the teeth is illustrated in figure 1, drawn from specimen 20784, collected by Dr. Whitehouse in Van Zandt County, Texas. A large part of the outer peristome is covered by the annulus which may be deciduous at times, but more often remains attached and must be removed to show the outer teeth fully. Figure 2 shows two teeth of the inner peristome



FIGS. 1 and 2. Peristome of *Clasmatodon parvulus* (Hampe) Sull.

from a different specimen, also from Texas, with well developed inner teeth. In attempting to make a mount which would show the base of the inner teeth, it was found that the outer and inner teeth are adherent, a point well illustrated in Sullivant's longitudinal section. The horizontal bars connecting elements of the endostome in ladder like fashion, are in fact, parts of the outer peristome, since they represent thickenings on the top and bottom walls of the cells. In the upper region the thickenings on the top and bottom walls are greater than on the vertical exterior walls of the cells. Halves of adjacent inner teeth are thus connected by thickenings which are usually a part of the outer teeth, and this explains why Wilson described the inner teeth as opposite the outer.

Since the description by Wilson (1842) seems adequate in most respects and more accurate than later descriptions, it is quoted below.

CLASMATODON (Hook. and Wils.). "Peristomium duplex. Exterius dentibus sedecim brevibus bipartibilibus bifidisve. Interius, membrana laciniis bifidis vel geminatis totidem iis suboppositis irregularibus devisa. Calyptra cuculliformis."

"CLASMATODON PUSILLUS (Hook. et Wils.), caule procumbente vage ramoso, foliis patentibus ovatis acutis seminervibus, capsula elliptica erectiuscula, operculo conico rostrato. Tab. XXV A"

"Hab. In arboribus prope New Orleans, Drummond."

"Similis Pterogonio decumbenti, Schwaegr. Suppl.; at rami non pinnatim dispositi. Caulis semiuncialis, rami ascendentes. Folia laxe imbricata, patientia, siccitate cauli adpressa, late ovata, acuminulata, integerrima, concaviuscula, nervo mediotenus instructa. Perichaetialia ovato-lanceolata. Seta laevis lineas tres vel quatuor longa. Capsula erectiuscula, oblongo-elliptica, ore annulo abscuro nec facile solubili cincto. Peristomii exterioris dentes valde obscuri, plerumque brevissimi, irregulares, aliquantulum torulosi, quandoque bifidi, rufescentes, interioris laciniae fulvae plerumque ad basin bifidae, cruribus inaequalibus. Operculum e basi convexa oblique brevi-rostratum. Calyptra dimidiata, glabra."

"This moss has been sent by Schweinitz from America, named *Pterogonium repens*. In size and general appearance it much resembles *Pterogon. subcapillatum*. The outer peristome is not easily detected but it is undoubtedly present."

In his illustration Wilson (1842) shows both outer and inner teeth, with the outer about half as long as the inner. In addition to the above description, some observations as to the papillose condition of the teeth might be added. The inner teeth vary from smooth to very strongly papillose, with all the intermediate stages present in different collections; and in well developed capsules the outer teeth may be strongly papillose at the tips. The key to the genera of the Fabroniaceae as given by Grout would be clearer if no. 3 were changed to read as follows;

3. Peristome double, teeth well developed, united in pairs, and reflexed when dry ..... 2. *Anacamptodon*

3. Peristome double with outer teeth much shorter than inner, and thus often appearing single, not reflexed when dry ..... 3. *Clasmatodon*

Clasmatodon is common in the southern states and was found once in Saxony, Germany, in 1851 by Bertram and was called *Anisodon Bertrami*. A small specimen of this collection from the Jaeger herbarium, is in the herbarium of the New York Botanical Garden, but only a few capsules are present. Inquiry of several European herbaria failed to locate any more of the European *Clasmatodon*. A mount made of a capsule from the Jaeger specimen showed both inner and outer peristome, and the leaves are similar to American specimens. Both Limpricht and Brotherus thought that the German and the American specimens were of the same species, and the observations made on available material confirm this.

It is interesting to note that in 1844, Hampe described this moss under

the name, *Rhegmatodon parvulus* and illustrated the peristome, which illustration resembles *Habrodon* rather than *Clasmatodon*. The genus *Rhegmatodon* of the Leskeaceae has a double peristome, with the outer teeth much shorter than the inner, but there is no indication in his description or illustration that Hampe saw the outer teeth of *Clasmatodon*. However, one wonders why he transferred the moss to this genus if he did not observe the outer teeth.

A study of other members of the Fabroniaceae, especially those with a single peristome such as *Ischyrodon, Dimerodontium, Fabronidium* and *Austinia* might yield new information, and the two latter genera are particularly interesting since they are described as having only an inner peristome. Observations of specimens of *Ischyrodon* and *Dimerodontium* in the herbarium of the New York Botanical Garden indicate that the peristome is an outer one, and no evidence of inner teeth was found, and in this they resemble *Fabronia* and *Habrodon*. No specimen of *Fabronidium* could be found, and although there were a number of collections of *Austinia tenuinervis*, the capsules were not too plentiful, or in very good condition; however, only one row of teeth was found. It is very desirable to get *Fabronidium* and more *Austinia* for careful study to determine whether there is any evidence of a double peristome.

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THE CULTIVATION OF TWO SPECIES OF ENTOMOPHTHORA ON SYNTHETIC MEDIA[1]

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The genera *Entomophthora* (*Empusa*) and *Massospora*, insect parasites belonging to the phycomycetous Order Entomophthorales, have long been regarded as obligate in their parasitism. According to Speare (1921) and Sawyer (1929), *Massospora* has never been grown in laboratory culture upon any artificial medium. In the case of *Entomophthora*, however, some 26 species of which are listed in the monograph of Thaxter (1888), several species have been grown successfully in the laboratory.

Brefeld (1884) reported growth of *Empusa muscae* Cohn in veal broth, in which medium the fungus produced mycelium, hyphal bodies, and conidiophores, but not conidia. Speare (1912) appeared to be somewhat skeptical of this claim because of the absence of corroboratory reports, since several investigators have attempted to cultivate this common species. Sheldon (1903), working with *Empusa grylli* Fresenius obtained from grasshoppers and caterpillars, was able to initiate cultures on bouillon agar from hyphal bodies and from conidia. Many attempts were required in order to yield pure cultures free of bacteria. Conidia were produced in Sheldon's cultures, one of which grew for 26 days.

Speare (1912) was unsuccessful in his efforts to cultivate *Entomophthora aulicae* Reichhardt (*Empusa grylli* Fresenius) from the browntail moth, *Euproctis chrysorrhoea*. Cultures were obtained, however, of a previously undescribed species designated as *Entomophthora pseudococci* Speare, isolated from the sugar cane mealy bug, *Pseudococcus calceolariae*. This fungus grew well upon a number of media, including potato, radish, oat agar, and potato agar, and infection of various insects after artificial cultivation of *E. pseudococci* was secured. Molliard (1918) isolated from *Culex pipiens* a fungus which he described as *Entomophthora Henrici* and which he was able to cultivate upon beef liver and upon carrot.

The most extensive studies concerning the laboratory cultivation of Entomophthorales which parasitize insects are those of Sawyer (1929). In addition to *Entomophthora pseudococci*, previously studied by Speare, Sawyer successfully cultivated *Entomophthora sphaerosperma* Fresenius from the black-headed fireworm, *Rhopobota vacciniana*, and an Empusa species from *Peronea minuta*. Some forty different media were found to support

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growth, including swordfish, pork, potato, and other natural media rich in proteins. It was concluded that neither carbohydrates nor fats were essential for growth. A pH of 6.5 was found to be optimal for these fungi. The only medium of strictly defined chemical composition employed by Sawyer was Czapek's agar, upon which growth of all three species was very poor.

It is the purpose of the present paper to record findings concerning the cultivation of two species, *Entomophthora apiculata* Thaxter and *E. coronata* (Cost.) Kevorkian, upon synthetic media of defined chemical composition. *Entomophthora apiculata* was described by Thaxter (1888) as an Empusa, parasitic upon various Lepidoptera, Diptera, and Hemiptera in Maine and North Carolina. The second species, *E. coronata*, was originally described by Costantin (1897) under the name *Boudierella coronata*, as a saprophyte upon the gills of mushrooms. The fungus was renamed *Delacroixia coronata* by Saccardo and Sydow (1899). It was next isolated as a saprophyte from orchid seeds by Gallaud (1905), who cultivated the organism upon various media, the most satisfactory of which was carrot, and infected roaches from these cultures. Gilbert (1919) isolated the same species from fern prothallia and from Sphagnum, and cultivated it upon potato agar, oatmeal agar, rice agar, pumpkin agar, and an agar medium containing beef extract.

Martin (1925) isolated from the fruiting body of a *Hypochnus* an organism which he described as *Conidiobolus villosus*, and which he cultivated upon prune decoction and a variety of other media. A second isolation of *C. villosus* was made by White (1937), during attempts to isolate ascospores of *Peziza domiciliana*, whereupon *C. villosus* was found as a contaminant upon plates of potato dextrose agar. It was shown by Kevorkian (1937) that *C. villosus* was identical with the previously described *Delacroixia coronata*. Further, Kevorkian demonstrated that this organism was a parasite of termites, and transferred the species to the genus Entomophthora. Couch (1939), although agreeing with the synonymy reported for this species, does not agree with the transfer of this organism to Entomophthora, but would prefer to retain the genus Delacroixia, pending the finding of zygospores. In the present report, the writer is arbitrarily following Kevorkian (1937) in designating the organism studied as *Entomophthora coronata*.

Materials and methods. The culture of *E. apiculata* employed in our studies was obtained through the courtesy of Dr. Kenneth B. Raper of the Northern Regional Research Laboratory, and is listed as NRRL 1626. Dr. Raper is of the opinion that this isolate represents Thaxter's type material of the species, since it was obtained from the culture collections of the Farlow Herbarium, Harvard University. The culture of *E. coronata* was obtained from the American Type Culture Collection, and is listed by them as number 10051. Stock cultures of these organisms were maintained upon slants of

potato dextrose agar at room temperature, and were transferred at intervals of 10–14 days. The sugars employed in our studies were obtained from the Pfanstiehl Chemical Company, while the amino acids were obtained from General Biochemicals, Inc.

Results. Two possibilities might be advanced in explanation of the failure of Sawyer (1929) to secure good growth of three Entomophthora species upon Czapek's agar; namely, the possibility that these organisms require vitamins or other accessory growth factors, and second, the possibility that they are unable to utilize the nitrate nitrogen present in Czapek's synthetic medium. Experiments were consequently designed to test these points with *E. apiculata* and *E. coronata*.

A medium, consisting of glucose, 30 gm.; *L*-asparagine, 2.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KH_2PO_4, 0.6 gm.; K_2HPO_4, 2.4 gm.; and double distilled water, to 1 liter; was prepared, adjusted to pH 6.0, sterilized by autoclaving, and dispensed aseptically into sterile, acid-washed 50 ml. Erlenmeyer flasks. The flasks were provided with metal caps rather than cotton plugs, to guard against possible contamination of the medium with vitamins from the cotton. Portions of the basal medium were supplemented with a stock vitamin mixture, containing thiamin hydrochloride, biotin, p-aminobenzoic acid, calcium pantothenate, inositol, nicotinamide, riboflavin, and folic acid, and to other portions no vitamins were added. The flasks were inoculated with mycelial fragments of *E. apiculata* and *E. coronata* from potato dextrose agar cultures, and maintained at room temperature. Within one week cultures of both species were growing nicely, forming mats across the surface of the medium, both with and without added vitamins, and discharging conidia, which adhered to the inner surfaces of the flasks, above the mats. Inoculum obtained from cultures without added vitamins was used to inoculate sub-cultures in the same media, in an effort to minimize by dilution the carry-over of vitamins with the inoculum. After five successive transfers, both *E. apiculata* and *E. coronata* appeared to grow as well without as with added vitamins. Another experiment involved the substitution of ammonium sulphate for asparagine as the nitrogen source, in order to rule out the possibility of contamination of the asparagine with vitamins. Both *E. apiculata* and *E. coronata* grew well in this medium, though the amount of growth was inferior to that obtained upon asparagine. No stimulation of growth was apparent upon the addition of vitamins. It may be concluded, therefore, that *E. apiculata* and *E. coronata* are autotrophic with respect to vitamins and other growth factors.

It was noted in the preceding experiment that ammonium nitrogen was inferior to amino nitrogen for growth of these organisms. In an effort to secure quantitative measurements of the suitability of amino, ammonium and nitrate nitrogen, the basal medium previously mentioned was modified

so as to include asparagine, ammonium sulphate, or sodium nitrate as the sole source of nitrogen, each in amounts calculated to provide 50 mg. N per 50 ml. After incubation for two weeks, the mats were harvested by filtration, placed on tared filter papers, and dried to constant weight over anhydrous calcium sulphate ("drierite"). Data obtained with *E. coronata* were as follows:

TABLE 1. *Utilization of various nitrogen sources by E. coronata.*

Nitrogen source	Mat weight	Final pH
L-asparagine	230 mg	6.4
$(NH_4)_2SO_4$	28	4.0
$NaNO_3$	0	6.0

Essentially similar findings were obtained with *E. apiculata*. It is apparent that while amino nitrogen is readily assimilated by these organisms, ammonium is a poor nitrogen source, and these two species of Entomophthora are unable to utilize nitrate nitrogen. A partial explanation of the poor growth upon ammonium sulphate is to be found in the lowering of pH of the medium to a level unfavorable for growth upon utilization of the ammonium ion.

Experiments with *E. coronata* designed to yield information concerning the relation of growth to pH were unsuccessful. The glucose-asparagine-salts medium was supplemented with McIlvaine's Na_2HPO_4-citric acid buffer, to provide a series of media adjusted to pH 3.5–8.0, at intervals of 0.5 pH unit. Growth occurred in all instances, and was better at pH 5.5–7.0 than elsewhere. After two weeks of growth the final pH of all cultures was determined and the mats were weighed. It was found that a pronounced shift in pH toward the alkaline side had occurred in all cultures, thus obviating the findings of the experiment. In view of the rather large shifts in pH accompanying growth in well buffered media, and the absence of a pronounced pH optimum, it was felt that this phase of the investigation should not be pursued further, and a pH of 6.0 was arbitrarily selected for all further experiments. It will be recalled that Sawyer (1929) regarded pH 6.5 as optimal for the species he studied.

The optimal glucose level for *E. coronata* was determined by experiments employing a basal medium containing 0.2% asparagine, with the glucose level varied from 0.1 to 10.0%. The results are presented in Table 2.

The glucose optimum is clearly within the range of 1–3 per cent, with the maximum mat weight being obtained in 2 per cent glucose. The decline in final pH with increasing levels of glucose is to be attributed to acid formation from glucose by the organism. When the culture filtrates were tested for

the presence of reducing sugars with Fehling's solution, no reducing sugar was detectable in cultures in which the original glucose concentration was 2 per cent or below. Thus, in these instances, the glucose was completely consumed within the two week period of incubation.

Using a glucose-salts basal medium, with a glucose level of two per cent, the optimal nitrogen level for growth was sought in a series of experiments in which the asparagine concentration was varied from 0.05 to 2.0 per cent. A pronounced growth maximum was obtained with 0.3 per cent asparagine, corresponding to 63.6 mg. N per 100 ml. Above this level, the mycelial mats were submerged, and the submerged growth habit was accompanied by a decrease in final pH and by limitation in growth.

The utilization of a number of sugars, polysaccharides, and alcohols as sources of carbon for *E. apiculata* and *E. coronata* was examined with the use of a 0.3 per cent ammonium sulphate-salts basal medium. To the basal medium were added various compounds as sole sources of carbon, each in

TABLE 2. *Results of growth of E. coronata in an asparagine-salts medium with varying amounts of glucose.*

Glucose concentration	Final pH	Mat Weight	Fehling Test
0.1%	8.1	50 mg	–
0.25%	7.9	71	–
0.5%	7.5	88	–
1.0%	6.4	345	–
2%	6.5	358	–
3%	6.4	351	+
4%	6.4	301	+
5%	6.2	266	+
7.5%	6.3	208	+
10%	5.5	91	+

an amount providing 400 mg. of carbon per 100 ml. The cultures were grown in 125 ml. Erlenmeyer flasks containing 33.3 ml. of medium, adjusted to an initial pH of 6.0. The experiment was carried out in triplicate. After growth for two weeks, the three cultures were pooled, and pH and mat weights were recorded. The data concerning mat weight therefore represent the sum of the mat weights of three cultures. The results are presented in Table 3:

From the data presented, it is apparent that *Entomophthora apiculata* and *E. coronata* are quite limited in their ability to utilize sugars and related compounds as sources of carbon. Among the substances tested, only glucose, levulose, galactose, mannose, and trehalose were utilized by these fungi. No qualitative differences were observed between the two species. Mannose, glucose, and trehalose would appear to be superior to levulose and galactose as sources of carbon. Pentoses, rhamnose, sorbose, disaccharides other than trehalose, trisaccharides, polysaccharides, and hexahydric alcohols were not

attacked. The utilization of hexose sugars and trehalose is accompanied by a marked degree of acid formation.

The utilization of some 20 amino acids as sources of carbon was examined in a similar experiment, employing an identical basal medium. In the case of the *L*-amino acids, the carbon level was set at 400 mg. carbon per 100 ml. as before. In the case of the *DL*-amino acids, this amount was doubled, on the assumption that the *D*-isomer is unavailable to the fungi. As in the preceding experiments, the pH was adjusted to 6.0. The results of experiments performed in triplicate are presented in Table 4, the figures for mat weight representing the combined weights of the mats in three replications of the

TABLE 3. *Growth of E. apiculata and E. coronata in media containing various sugars, polysaccharides, and alcohols as sole sources of carbon.*

Carbon source	*E. apiculata*		*E. coronata*	
	Mat weight	Final pH	Mat weight	Final pH
D-Arabinose	0 mg	6.0	0 mg	6.0
Xylose	0	6.0	0	6.0
Rhamnose	0	6.2	0	6.0
Glucose	105	3.6	96	3.5
Levulose	36	4.5	68	3.7
Galactose	22	5.7	40	4.6
Mannose	121	3.6	134	3.2
Sorbose	0	6.0	0	6.0
Sucrose	0	6.0	0	5.8
Maltose	0	6.0	0	5.8
Lactose	0	6.0	0	5.8
Cellobiose	0	5.8	0	6.0
Melibiose	0	5.8	0	6.0
Trehalose	78	3.4	117	3.3
Raffinose	0	6.0	0	6.0
Melezitose	0	6.0	0	6.0
Dextrin	0	6.0	0	6.0
Soluble starch	0	6.1	0	6.0
Sorbitol	0	6.0	0	6.0
Mannitol	0	6.0	0	6.0
Inositol	0	6.0	0	6.0

experiment. For purposes of comparison, the data obtained with glucose in a previous experiment (Table 3) are also included.

It is apparent that in general, the amino acids tested are inferior to glucose as sources of carbon. Alanine, aspartic acid, and tyrosine are comparable to glucose as carbon sources for *E. coronata*, but no amino acid approached the effectiveness of glucose in promoting growth of *E. apiculata*. The results obtained with amino acids as carbon sources for *E. apiculata* are quite different from those obtained with *E. coronata*. Phenylalanine, methionine, tyrosine, aspartic acid, and proline were the most favorable carbon sources for *E. apiculata*, while alanine, tyrosine, aspartic acid, asparagine, and glutamic acid induced the most growth of *E. coronata* under these con-

ditions. Neither fungus was able to utilize cystine, cysteine, threonine, isoleucine, or norleucine as a source of carbon, while *E. apiculata* was likewise unable to assimilate carbon from valine.

Further experiments were performed to examine the suitability of 24 different amino acids as sources of nitrogen for *E. apiculata* and *E. coronata*, using a two per cent glucose-salts basal medium. The various amino acids were added in quantities providing 31.8 mg. of nitrogen per 100 ml. In the

TABLE 4. *Growth of E. apiculata and E. coronata in media containing various amino acids as sole sources of carbon and as sole sources of nitrogen.*

Amino acid	Growth in medium containing amino acid as sole source of carbon		Growth in medium containing amino acid as sole source of nitrogen	
	E. apiculata Mat weight	*E. coronata* Mat weight	*E. apiculata* Mat weight	*E. coronata* Mat weight
Glycine	18	27	498	414
beta-Alanine			16	35
L-Aspartic acid	66	93	468	498
L-Glutamic acid	48	81	411	153
L-Asparagine	30	84	545	449
L-Cystine	0	0	120	75
L-Cysteine. HCl	0	0	163	71
L-Leucine	48	28	105	33
L-Lysine. HCl	12	18	44	36
L-Histidine. HCl	30	24	258	104
L-Arginine. HCl	6	23	84	90
L-Tryptophane	6	26	26	15
L-Tyrosine	75	95	491	77
L-Proline	54	27	171	60
L-Hydroxyproline			13	20
DL-Alanine	51	116	474	363
DL-Serine			556	173
DL-Threonine	0	0	83	101
DL-Valine	0	15	147	50
DL-Isoleucine	0	0	54	21
DL-Norleucine	0	0	27	9
DL-Phenylalanine	87	26	182	18
DL-Ornithine. HCl			266	59
DL-Methionine	75	20	133	53
Glucose control	105	96		

case of the *DL*-amino acids, this quantity was doubled, on the assumption that the *D*-isomer is probably unavailable to the fungus. The mats were weighed after incubation for two weeks. The results obtained are also included in table 4.

Serine, asparagine, glycine, tyrosine and alanine proved to be the five best amino acids for growth of *E. apiculata*, while, for *E. coronata* the best were aspartic acid, asparagine, glycine, alanine and serine. All 24 amino acids tested were utilized to some extent as sources of nitrogen by both fungi,

even though a number of these were apparently unavailable to these same species of Entomophthora as sources of carbon.

Discussion. The experiments just described would appear to indicate that *Entomophthora apiculata* and *E. coronata*, both of which species are parasites of insects, can readily be grown in a variety of synthetic media. Prior to the work of Sheldon (1903), Speare (1912), Molliard (1918) and Sawyer (1929), all species of Entomophthora were considered to be obligate parasites. The present report is believed to represent the first instance in which a parasitic species of the Entomophthorales has been cultivated on a medium of defined chemical composition. Although the species studied by Sawyer (1929) are not identical with the species upon which the present study is based, it would appear to be a reasonable inference that the failure of Sawyer to secure good growth of *Entomophthora pseudococci*, *E. sphaerosperma*, and *Empusa* sp. on Czapek's agar is probably due to the inability of these forms to utilize nitrite as a source of nitrogen.

Insofar as the writer is aware, no species of the related genera Massospora, Completoria, or Ancylistes has been cultivated as a saprophyte, (Speare, 1921; Couch, 1939). The situation is somewhat different in the genus Conidiobolus, however. Brefeld (1884) grew *C. utriculosus* on dung decoction. *Conidiobolus Brefeldianus* originally was discovered as a contaminant upon a plate of maltose-peptone agar by Couch (1939), who has grown this species upon a number of materials of organic origin, including cooked carrots and sweet potatoes, egg yolk, and potato dextrose agar. Conidiobolus has not, however, been grown in synthetic media.

The only member of the Entomophthorales which has been previously cultivated in synthetic media is the saprophytic species, *Basidiobolus ranarum*. The studies of Leonian and Lilly (1938) indicate that this organism does not require thiamin or other accessory growth factors. It is unable to utilize nitrate or ammonium nitrogen, but can be grown upon a medium in which the nitrogen is provided as a mixture of amino acids. In subsequent experiments with single amino acids, it was found that *B. ranarum* made excellent growth in a medium containing 0.1 per cent arginine, and fair growth in the presence of aspartic acid, glutamic acid, glycine, alanine, phenylalanine, histidine, valine, leucine, or serine as sole sources of nitrogen. Except for the fact that *E. apiculata* and *E. coronata* can utilize ammonium nitrogen, while *B. ranarum* cannot, similarities in the nutrition of these three species are more apparent than are differences.

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SUMMARY

1. Insect-parasitizing species of the genus *Entomophthora* have not previously been grown in synthetic media of defined chemical composition. It was found that *Entomophthora apiculata* Thaxter and *E. coronata* (Cost.) Kevorkian can be grown upon a glucose-asparagine-salts synthetic medium. Both species are autotrophic with respect to vitamins and other accessory growth factors.

2. These fungi cannot assimilate nitrate, but utilize ammonium or amino nitrogen. The optimal glucose concentration for growth of *E. coronata* is 2.0%; the optimal asparagine concentration is 0.3%. *E. apiculata* and *E. coronata* are quite restricted with respect to the utilization of sugars as sources of carbon; of a number of sugars tested, only glucose, levulose, galactose, mannose, and trehalose were utilized. The utilization of these hexose sugars and trehalose is accompanied by acid formation. A number of amino acids, including cystine, cysteine. HCl, threonine, isoleucine, and norleucine are unavailable to these two species as sources of carbon.

3. Growth of both *E. apiculata* and *E. coronata* occurred with each of 24 amino acids tested as sole sources of nitrogen. Serine, asparagine, glycine, tyrosine, and alanine were the most favorable nitrogen sources for *E. apiculata*, while aspartic acid, asparagine, glycine, alanine, and serine were best in supporting growth of *E. coronata*.

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THE RESTING NUCLEUS IN PHYSARIA AND LESQUERELLA

SOPHIE JAKOWSKA

The condition of chromatin in the resting nuclei of plants has been studied by numerous investigators since prochromosomes were first reported by Overton (1909) and Rosenberg (1909). A history of the subject has been given by Doutreligne (1933), Smith (1934) and Flavin (1943). A study of the papers included by these authors showed however that chromatin bodies in resting nuclei, regardless of size and number, have often been designated as prochromosomes.

In a previous study (Jakowska 1949), the writer reported prochromosome-like bodies in cells of *Physaria Geyeri, P. australis* and *Lesquerella Sherwoodii.* It was mentioned that the number, size and other characteristics of these bodies showed no relation to the diploid number of chromosomes. The purpose of the present paper was to ascertain whether or not the chromatin bodies reported for the above plants were true prochromosomes, and in addition to determine if they play a part in cellular differentiation as was suggested by Huskins and Steinitz (1948) for similar structures in the resting nuclei from the roots of *Rhoeo.* The term "heterochromatic body" employed by the latter authors has been accepted for the chromatin bodies described in this paper.

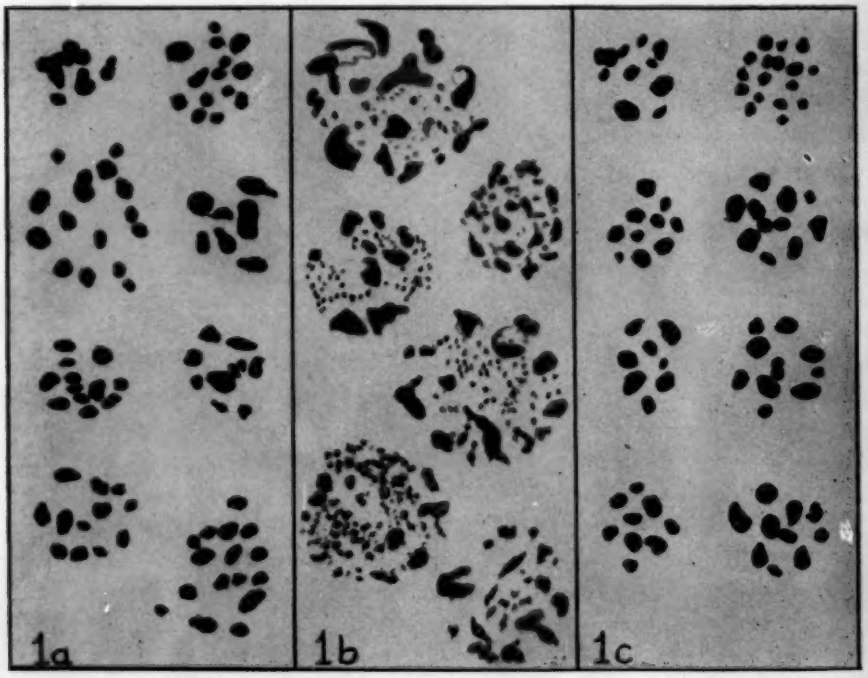
Material and methods. The plants, including *Physaria Geyeri, P. australis* and *Lesquerella Sherwoodii,* were obtained through the courtesy of Dr. Bassett Maguire, Curator of the New York Botanical Garden. They were raised to maturity from seeds in the greenhouse of the New York Botanical Garden.

The cytological studies were carried out on Feulgen-stained smear preparations from leaves of mature plants of the three species and from various parts of 6-week-old *P. Geyeri,* such as cotyledons, first leaves, leaf petioles, stems, primary and secondary roots. Sections of the young plants were stained with iodine and gentian violet. Drawings were made with the help of a camera lucida with 15x ocular and 98x oil immersion objective.

Observations. Heterochromatic bodies were counted in 100 nuclei selected at random from cells of each of the different tissues of 6-week-old *P. Geyeri.* The numbers per nucleus were as follows: cotyledons (fig. 3) 9 to 12; mesophyll and epidermis of young leaves 6 to 17; petioles 7 to 21; young trichomes in the late rosette-like stage of differentiation (fig. 4, Jakowska 1949) 9 to 16 (fig. 4); primary roots (fig. 5) 8 to 21 and secondary

roots (fig. 6) 13 to 30. In addition, the degree of vacuolization and the intensity of the Feulgen reaction varied in the individual bodies within the same nucleus and among the bodies of the nuclei from different tissues (figs. 3 to 6).

Nuclei with finely divided chromatin in addition to typical heterochromatic bodies occurred constantly in the parenchyma cells of the primary roots, a condition which also occurred, with lesser frequency, in the nuclei of the secondary roots. An occasional nucleus of this type was also seen in the



FIGS. 1–6. FIGS. 1a–1c. Chromatin contents of nuclei from young leaves of adult plants of *Physaria Geyeri* (1a), *P. australis* (1b) and *Lesquerella Sherwoodii* (1c). Feulgen-stained smears. × 2200.

cells of the cotyledons and more rarely among the trichomes. Such nuclei, however, were not encountered in the cells of the young and mature leaves and petioles in *P. Geyeri* or *L. Sherwoodii* (figs. 1a and 1c).

It is of interest to notice that the condition of the nuclei in the roots of *P. Geyeri* (fig. 5) resembled that of the nuclei in the leaves of *P. australis* (fig. 1b) since in both cases finely divided chromatin in addition to the heterochromatic bodies was seen. The observed diploid number of chromosomes in *P. australis* was 2n = 8, like in *P. Geyeri* (fig. 2), although the gross appearance and growth habit of the plant suggested the possibility of poly-

ploidy. The cells and the nuclei of *P. australis* were, however, larger than those of *P. Geyeri* and the chromosomes at late prophase and metaphase differed in shape, size and stainability.

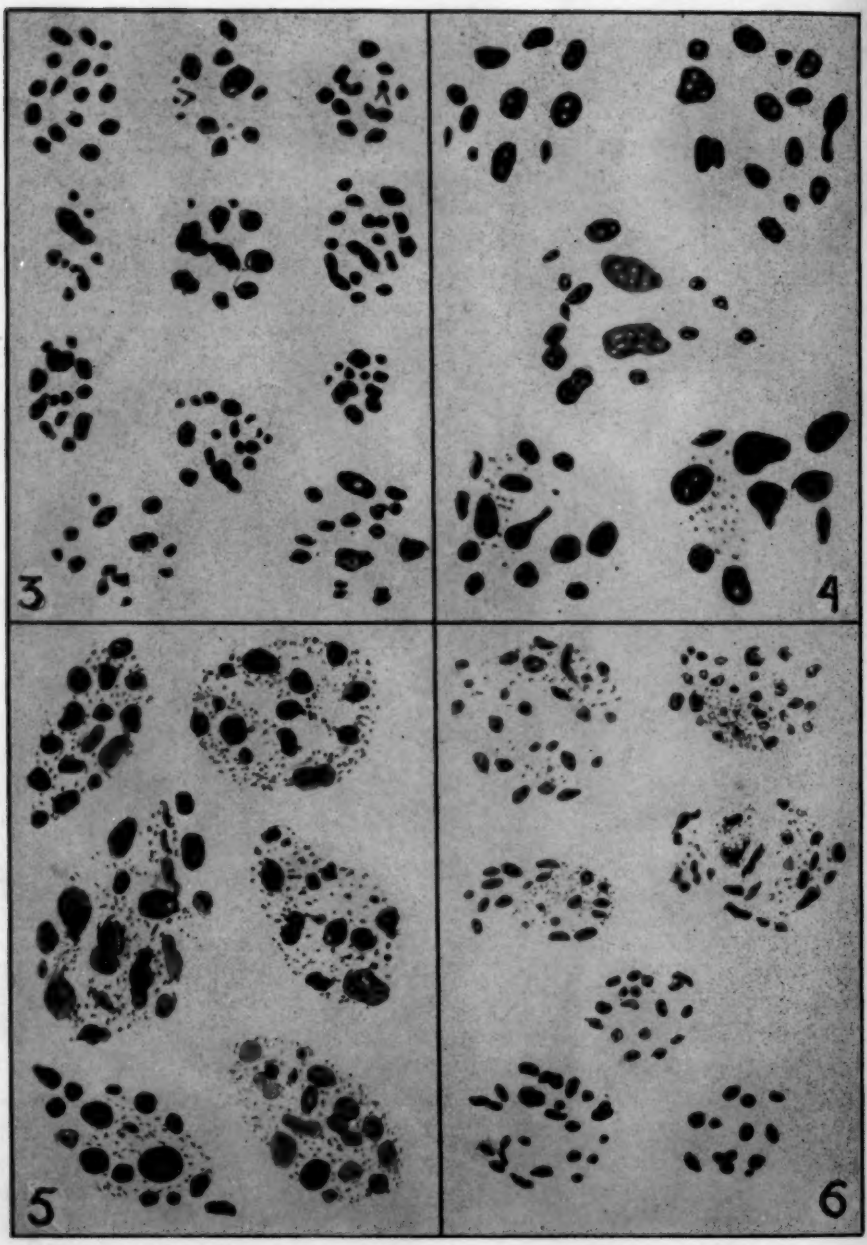
An attempt was made to trace the heterochromatic bodies through the mitotic cycle in *P. Geyeri*. The emergence of the chromosomes in prophase did not indicate that definite heterochromatic bodies participated in the formation of each chromosome. Occasionally a slightly Feulgen-positive tube-like structure was observed connecting two heterochromatic bodies but threads arising from heterochromatic bodies or connecting fibers between

FIG. 2. Metaphase plate from the leaf meristem of *P. Geyeri* (2n = 8). Feulgen-stained smear. Greatly enlarged.

them were not seen. The transition in late telophase to heterochromatic regions and bodies indicated that several heterochromatic bodies may arise from each chromosome.

Discussion. In the present study on *P. Geyeri* both meristematic and differentiated cells were examined. In the resting cells from meristematic regions the number of heterochromatic bodies was not constant and hence they are not to be considered as prochromosomes. The use of the latter term should be restricted to those cases in which a relationship exists between the heterochromatic bodies in the resting nucleus and the chromosomes at mitosis.

In the differentiated cells of *P. Geyeri* it was not possible to establish any regular pattern for the shape and number of heterochromatic bodies.



FIGS. 3–6. Chromatin contents of nuclei of 6-week-old *P. Geyeri* from cotyledons (fig. 3), from developing trichomes (fig. 4), from primary roots (fig. 5), and from secondary roots (fig. 6). Feulgen-stained smears. ×2200.

As a rule the smaller nuclei contained smaller and more numerous heterochromatic bodies while in comparatively larger nuclei fewer but larger bodies were found. Observations on the nuclei of the trichomes in different stages of development found on the 6-week-old plants revealed that during the process of growth and differentiation the number of heterochromatic bodies remains relatively constant. Polyploidy in the fully developed trichomes, suggested previously (Jakowska 1949) on the basis of the increased nuclear and nucleolar volumes, appears to be related to the size rather than to the number of heterochromatic bodies. On the other hand, Huskins and Steinitz (1948) observed that in the roots of *Rhoco* differentiation was accompanied by an increase in number of heterochromatic bodies attributable to polyploidy.

The comparison of the nuclei in the corresponding regions of leaves from adult *P. Geyeri, P. australis* and *L. Sherwoodii* suggested a greater similarity between *P. Geyeri* and *L. Sherwoodii* than between the two species of the genus Physaria. This, together with the findings on the adult trichomes of the three species (Jakowska 1949) may furnish further clues as to their taxonomical relationships.

The varied response to the Feulgen reaction exhibited by the heterochromatic bodies within the same nucleus suggested differences in the desoxyribosenucleic acid content, but it is most unlikely that such differences could be validly demonstrated by quantitative methods (Ely and Ross 1949).

SUMMARY

1. The nuclei in the leaves of adult *P. Geyeri* ($2n = 8$) and of *L. Sherwoodii* differed from those of *P. australis* ($2n = 8$) in possessing finely divided chromatin in addition to the characteristic heterochromatic bodies.

2. In 6-week-old *P. Geyeri* most resting nuclei in the primary root and some nuclei in the secondary roots were also in this condition, but in the nuclei of cells from cotyledons, young leaves, petioles and developing trichomes the finely divided chromatin was absent.

3. The number of heterochromatic bodies in *P. Geyeri* was not related to the diploid number of chromosomes, or multiples thereof, indicating that they are not prochromosomes.

4. Individual heterochromatic bodies within the same nucleus and those from cells of various parts of the plants differed in their response to the Feulgen reaction.

5. An increase in the size of individual heterochromatic bodies, rather than an increase in their number, was noticed in differentiating stellate trichomes of *P. Geyeri*.

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AMINO ACID TOXICITIES TO TOBACCO VARIETIES DIFFERING IN RESISTANCE TO BLACK ROOT-ROT

ROBERT A. STEINBERG

The factors underlying disease resistance in plants are still more or less obscure. That they are definitely of genetic origin is clear from the work of the plant breeder. Nevertheless little is as yet known concerning the chemical factors underlying resistance in plants. It is known, however, that exosmosis of many metabolic compounds ordinarily occurs from microorganisms, and that these include amino acids, enzymes, toxins, etc. Since the vast majority of microorganisms can be cultured on dead materials, it may be assumed that any harmful effects of microbial exosmosis on the green plant are ordinarily beneficial to the pathogen. From a biochemical viewpoint the situation can be viewed most simply as the interaction of two organisms mutually immersed in each others enzymes and metabolites.

The discovery that many free amino acids are rather toxic to tobacco seedlings in aseptic culture by the writer (1947, 1949) has made it desirable to determine whether any degree of correlation exists between varietal susceptability to injury by free amino acids and disease resistance. Free amino acids are normal constiutents of the plant as well as the microorganism and their presence and fluctuations in concentration may be a factor in disease resistance. A study of varietal differences would in itself be of some value for the purpose of disclosing the extent of variation within a species. Six varieties of tobacco were selected for this survey on the basis of recommendations made by Dr. E. E. Clayton of this Bureau. Three of these are considered by plant pathologists to be susceptible and three resistant to black root-rot (*Thielaviopsis basicola* (Berk. & Br.) Ferr.). The former included Maryland Medium Broadleaf, Havana 38, and Judy's Pride; the latter, Havana 142, Havana 307, and Harrow Velvet.

Experimental methods. The seedlings were grown in duplicate on 50 ml. of a 2% sucrose mineral agar after germination in sterile petri dishes. Pyrex Erlenmeyer flasks of 200 ml. capacity were employed. Sterilization of seed was for 15 minutes with 1 : 1000 silver nitrate solution. A temperature of 25° C . and approximately 500 foot-candles of light furnished by white fluorescent lamps permitted rapid growth of the seedlings. Growth was for 4 weeks and was scored as 0, 1, 2, 3, 4 or approximately 0, 10, 25, 50, and 100 per cent. Toxicity of amino acids was tested at a level of 40 mg. per liter, i.e. 40 p.p.m. With the exception of methionine, ornithine, serine, and threonine, all amino acids used were the natural or levo-isomers. The four

enumerated were racemic mixtures. They were added to the medium prior to sterilization at 15 pounds pressure for 30 minutes. Additional details of technique will be found in a previous paper by the writer (1947).

Experimental results. Examination of table 1 will disclose that each amino acid was toxic to one or more varieties of tobacco even at the low level of 40 p.p.m. Arginine was the sole exception. Further study of the table will also reveal that with the exception of arginine, isoleucine, and glutamic acid, all were more toxic on the whole to the susceptible tobacco varieties. Arginine was innocuous to all at this level, isoleucine was less toxic to two susceptible varieties, and glutamic acid to one.

TABLE 1. — *Relative seedling growth with free amino acids at 40 p.p.m. of six tobacco varieties susceptible and resistant to black root rot.*

I	IV A
Non-toxic to all varieties	More toxic to one susceptible variety
Arginine	Cystine
	Glutamic acid
II A	Glycine
Slightly toxic to all varieties	Histidine
Glutamic acid	Leucine
Glycine	Ornithine
Isoleucine*	Phenylalanine
Methionine	Proline
	Tryptophane
II B	Tyrosine
Toxic to all varieties	IV B
Alanine	More toxic to two susceptible varieties
Hydroxyproline	Aspartic acid
Serine	Lysine
	Serine
III	Valine
Slightly less toxic to a susceptible variety	IV C
Isoleucine	More toxic to all susceptible varieties
Glutamic acid	Methionine
	Threonine

* All varieties frenched but Havana 38 and 142 produced extreme symptoms.

The intermediate amino acid toxicity level of 40 p.p.m. had been selected on the basis of previous studies with the Maryland variety. Differential responses as between susceptible and resistant varieties tend therefore to be minimized with such amino acids as arginine, cystine and ornithine because of low toxities. The converse is also true, particularly for hydroxyproline, which is lethal for the Maryland variety at 5 p.p.m. The level chosen was considered to be a fair approximation of that probably occurring in exudates from fungous hyphae and germinating spores.

Another interesting feature of these data was the emergence of what may be a species response. Tobacco varieties as a group appeared to be sensi-

tive to the action of minute quantities of alanine, hydroxyproline, and serine.

A comparison was made of the quantitative data by comparing the results with five of these varieties with those for the Maryland. Havana 38 was more sensitive to the action of seven amino acids; Judy's Pride to five; Havana 142 and 307 to alanine and isoleucine; and Harrow Velvet only to isoleucine. On the other hand Havana 38 was less sensitive than Maryland to eight amino acids, Judy's Pride to three, Havana 142 and 307 to eleven and Harrow Velvet to eight. Harrow Velvet is probably the variety most resistant to black root-rot and Judy's Pride the least, or one of the least.

Fourteen of the twenty amino acids were injurious to the Maryland variety, fourteen to Havana 38, eleven to Judy's Pride, six to Havana 142, six to Havana 307, and eight to Harrow Velvet. Susceptible varieties, therefore, appeared to be susceptible to injury by a greater number of amino acids than were the resistant varieties.

The average growth score of each of the varieties was also computed for the twenty amino acids. The three susceptible varieties (Maryland Medium Broadleaf, Havana 38, and Judy's Pride) gave values of 2.75, 2.80, and 2.65. Those of the resistant varieties (Havana 142, 307, and Harrow Velvet) gave ratings of 3.40, 3.40, and 3.20. Scores of all susceptible varieties averaged 2.73 ± 0.08; and resistant varieties 3.33 ± 0.13. The non-resistant varieties were therefore more susceptible to the toxic action of traces of free amino acids than were the resistant.

Most of the differences obtained in these scores were due to the marked toxicity of serine, threonine and valine to susceptible tobacco varieties. The relative scores of these six tobacco varieties for these three amino acids were 2, 2, 1.67, and 3.67, 3.67, 2.67, respectively. The average scores for susceptible and resistant varieties for these three amino acids were therefore 1.89 and 3.34. Attention is called to the fact that averages of the scores tend to minimize the growth differences between susceptible and non-susceptible varieties as compared to averages of the percentage growth.

The effects of products of fungal and bacterial exosmosis were also studied with tobacco seedlings in aseptic culture. Growth of the fungi used on the mineral agar was as numerous fine, transparent hyphae. Infection of the seedling occurred with *Rhizoctonia solani* Kuehn. *Sclerotium rolfsii* Sacc., *Thielaviopsis basicola* (Berk. & Fr.) Ferr. and *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker. The presence of 2% sucrose increased the degree of infection with *R. solani, Pythium irregulare* Buis., *T. basicola,* and *Fusarium oxysporum* Schlecht. var. *nicotianae* J. Johnson. These responses may have been due partially or wholly to larger inocula because of greatly increased growth of the fungi however. The presence of 200 p.p.m. of peptone did not increase fungous growth as greatly but still gave similar responses with *R. solani, P. irregulare,* and *P. parasitica.*

Repetition of these experiments under better controlled conditions of size of inocula would seem desirable.

Though *Cerocospora nicotianae* Ell. & Ev. grew in the medium in the absence of sugar and peptone the seedlings remained healthy and vigorous. The major difference consisted in the roots being red due to absorption of color from the fungus. This fungus causes a tobacco leaf spot and has not been reported on the roots of its host. Nevertheless infection of tobacco occurred on injuring the roots during inoculation.

Other data illustrative of the effects of exosmotic products of microorganisms on disease were obtained with *Bacterium angulatum*[1] Fromme & Murray and *Bacterium tabacum*[2] Wolf & Foster. Inoculation of the agar medium to which two percent sucrose had been added was without effect with the former organism. The presence of *B. tabacum* resulted, however, in a marked chlorosis through the centers and bases of the leaves and the formation of necrotic spots. *B. tabacum* forms a toxin according to Clayton (1934).

An asymmetrical inhibition of root growth was obtained with most of these pathogens when the medium was infected marginally at one point. The roots of the seedling growing towards the point of inoculation were usually stunted as compared to those growing in other directions. Asymmetrical inhibition of root growth was least with *Thielaviopsis* on addition of sucrose to the medium.

The presence of harmful diffusion products from the hyphae of these fungi must be assumed on the basis of these responses. Asymmetrical stunting of seedling roots was often visible before contact of fungus and root had been established. None of these fungi have as yet been identified as producers of toxins, excepting *F. oxysporum* (Wolf and Wolf, 1948).

Discussion. It is well known of course that many pathogenic microorganisms cause inactivation or death of the host cells in advance of penetration and so facilitate invasion of the tissues. Certain of these organisms do so through liberation of toxins. The mechanism whereby the majority act has been unknown. Nevertheless it can be accepted as demonstrated that exosmosis of metabolites ordinarily takes place from cells of growing microorganisms, and even from ungerminated spores (Wain 1948) in the presence of moisture. These data therefore comprise a demonstration of a similar toxin-like action of ordinary fungous metabolites like the amino acids. Its basis is the difference in toxicity of certain microbial metabolites to the microorganism and its host. Hydroxyproline, for example, is lethal to tobacco seedlings at 5 p.p.m. (Steinberg 1950) and harmless to most fungi in much higher concentrations. Dr. F. C. Steward has informed the writer

[1] *Pseudomonas angulata* (Fromme & Murray) Holland in Bergey's Manual.
[2] *Pseudomonas tabaci* (Wolf & Foster) F. L. Stevens in Bergey's Manual.

that free hydroxyproline was not encountered by him in amino acid examinations of green plants by paper chromatography. It would seem probable from these results that the toxicities of the amino acids diffusing from the hyphae of the black root-rot fungus are a factor in overcoming the resistance of tobacco plants to infection. Once infection has taken place, moreover, an increase in diffusion products is to be anticipated. These effects need not of course be displayed on an extensive scale to be effective as an aid in infection and invasion. Partial immobilization of successive cells of the host would produce a better result so far as the fungus is concerned than would extensive killing of the host cells.

Supplying sucrose and peptone to the medium was often effective in increasing infection. This result was due presumably to an increase in quantity of fungal products of exosmosis, as well as increased vigor of the fungi. Increased growth of the fungus also meant increased inocula as well as increased diffusates. The addition of sucrose and peptone are ordinarily beneficial for growth of tobacco seedlings in the absence of pathogenic organisms.

The ability of certain microorganisms to cause infections has been associated with the capacity to produce toxins. Virulent strains losing the ability to produce toxins become avirulent. It is quite possible that loss in capacity for the formation of certain of the amino acids, a well known phenomenon in fungi, may afford a partial explanation in some cases of lost pathogenicity.

A comparison by chromatographic analysis of the concentration of each of the free amino acids in exudates of fungous hyphae and germinating spores with varietal sensitivity to amino acid action should be most revealing when correlated with disease susceptibilities. Varietal and strain differences in the two organisms would, it is almost certain, necessitate special tests in each individual instance. The magnitude and complexity of these studies are evident since previous data have shown that the free amino acids in mixture may modify each other's effects on the plant. These mutual effects depend on the particular amino acids used and on their relative concentrations. Nevertheless the general survey here presented discloses the existence of a positive correlation between sensitivity to amino acid toxicity and disease susceptibility of tobacco to black root rot.

SUMMARY

1. Amino acid toxicities to three varieties of tobacco susceptible to black root rot (*Thielaviopsis basicola* (Berk & Br.) Ferr.) and to three resistant to this fungus were tested in aseptic culture.

2. The amino acid level employed was 40 p.p.m. The susceptible varieties were Maryland Medium Broadleaf, Havana 38, and Judy's Pride. The

resistant varieties were Havana 142, Havana 307 and Harrow Velvet.

3. The seedlings were grown for four weeks at 25° C. with white fluorescent light of about 500 foot-candles.

4. All six varieties of tobacco seemed to be specially sensitive to the action of alanine, hydroxyproline, and serine.

5. The average growth score was 2.73 for susceptible varieties, and 3.33 for resistant varieties with all 20 amino acids.

6. Isoleucine produced symptoms of frenching in all varieties.

7. Serine, threonine, and valine were definitely more toxic to susceptible varieties. The growth scores for these three were 1.89 and 3.34, respectively.

8. In general, toxicity to the host cells of free amino acids showed a positive correlation with susceptibility to black root rot. Presence of free amino acids in fungous exudates was concluded to be a factor in overcoming resistance of the plant to disease and facilitating invasion of host tissues.

9. Ordinary metabolites of the fungus, according to this view, can therefore exercise a localized toxin-like action on the host plant when a marked difference exists in their toxicity to host and pathogen.

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NATURAL HYBRIDIZATION IN THE GENUS CIRSIUM—I C. DISCOLOR (MUHL. EX WILLD.) SPRENG. X C. MUTICUM MICHX.

GERALD B. OWNBEY[1, 2]

The occurrence of natural hybridization among species of North American thistles is a subject still very incompletely studied. According to Petrak (1917), however, natural hybridization among European thistles has been known for many years and at the time of his writing, he states, the number of known hybrids in Europe was about three times greater than the number of species. In the *Cirsium* literature of America, there seems to be no earlier mention of putative interspecific hybridity than that pointed out by Petrak, viz. the observations of Asa Gray published over seventy-five years ago. Gray (1874) in his description of *Cnicus Parryi* suggested that a Colorado specimen, Hall and Harbour No. 341 "is probably a hybrid of this with *C. eriocephalus.*" On the following page (l.c., p. 48) under *Cnicus carlinoides* Schrank var. *Americanus* (later *Cirsium americanum*) a Kellogg specimen from Mendocino County, California is referred to as "possibly a hybrid with *C. remotifolius.*" Gray (1880) refers a second time to putative hybridization between *Cnicus carlinoides* var. *Americanus* and *C. remotifolius* in the Botany of California where he notes in another connection that Hall and Harbour's No. 559 (in Gray's Syn. Fl., p. 399, corrected to read Kellogg & Harford No. 559) "looks very much like a hybrid between *C. carlinoides* and *C. remotifolius.*" The putative hybridization between *Cnicus Parryi* and *C. eriocephalus* is once more referred to by Gray in his Synoptical Flora (1884) in the following manner: "Appears to hybridize with *C. eriocephalus,* & c." Further, Gray in discussing *Cnicus americanus* makes the following note: "A hybrid with *C. undulatus?* with red-purple flowers and purplish tips to involucral bracts, is from Pinos Altos Mountains, New Mexico, Greene."

Thus it appears that the possibility of hybridization involving at least five western species of *Cirsium* was clearly recognized by Gray. Whether or not these reports really represent true natural hybrids has not yet been demonstrated.

[1] This investigation was supported in part by a research grant from the Graduate School, University of Minnesota. The field work was carried out during the summer of 1949 from the University of Minnesota Forestry and Biological Station, Itasca State Park, Minnesota.

[2] The writer wishes to express his thanks to Dr. Edgar Anderson for critical reading of the manuscript. Dr. Thomas Morley has been of material assistance in checking the chromosome counts.

Putative interspecific hybridity was fully exploited by P. A. Rydberg (1910) in his interpretation of morphologically intermediate specimens of North American thistles. Nineteen putative hybrids involving twenty-one species recognized by himself were described, primarily from Colorado, but also from Utah, Wyoming, Montana, Kansas and Nebraska. It is noteworthy that most of Rydberg's proposed hybrids were described from specimens collected by other botanists, notably by G. E. Osterhout. Their validity can hardly be accepted until supported by data from field studies and experimentation.

Petrak (1917) published his excellent but incomplete treatise on the North American species of the genus *Cirsium* in 1917. He briefly reviewed the previous literature on hybridity in the genus and described five additional putative hybrids involving seven species principally from California, but two from the Turtle Mountains of North Dakota. The last, a hybrid between *C. muticum* and *C. canescens* is of special interest because it is the first report of a presumed hybrid which involved one of the parents of those described in this paper.

Later floristic publications of Rydberg (1917, 1932) continued to recognize natural hybridization in *Cirsium*, but his observations have not since been corroborated by other workers.

Extensive field work on the midwestern species of *Cirsium* was undertaken as a preliminary to a systematic revaluation of the species of that region. The preliminary observations reported here are from an attempt to investigate the importance of hybridization in *Cirsium*, first in the obliteration or obscuring of species lines, and secondly in the evolution of the genus.

The first collection of a putative hybrid between *C. discolor* and *C. muticum* was made 1.8 miles south of Plummer, Red Lake County, Minnesota (*G. B. Ownbey and Findley Ownbey No. 1209*, August 7, 1949). The single plant was growing in proximity to both parental species in a zone intermediate between them. Since the populations of the species were not sympatric, and since the conditions at this locality paralleled those at a second locality later discovered, no further details will be enumerated here. A careful examination of about forty plants of *C. discolor* and about twelve plants of *C. muticum* in the vicinity was made but no further evidence of hybridization was detected.

A second and more extensive occurrence of putative hybrids between the two species was discovered a few days later 13.5 miles west of Bagley, Polk County, Minnesota (*G. B. Ownbey and Findley Ownbey No. 1231a, b, and c*, August 17 and 20, 1949). A total of about forty plants was counted which were interpreted as ultimately of hybrid origin. There were approximately two hundred plants of *C. discolor* in the vicinity and also several hundred

plants of *C. muticum*. After a preliminary analysis of the species and hybrids in the field, specimens were collected for laboratory analysis of variation.

Ecologically and taxonomically, these two species of *Cirsium* are well separated. *C. discolor* is a species of eastern and central United States. Its westward distribution, at least in Minnesota, is limited by the transition zone between deciduous forest and prairie. It occurs in open mesophytic environments, usually in fairly well drained, light, often disturbed soils. *C. muticum*, as its common name "swamp thistle" indicates, is most commonly found growing in highly organic, poorly drained soils, often at the borders of bogs, drainage ditches and in wet, open meadows. Rarely does it invade the drier areas around its natural habitats. *C. muticum* is most characteristically found in the eastern conferous forest zone. Its most western occurrence in the United States is in the Turtle Mountains of North Dakota. Distribution maps of *C. discolor* and *C. muticum* indicate a wide north-south overlap in ranges. These maps, however, do not express the very distinct habitat optima of the two species, even when growing in the same geographical area.

The occurrence together of the two species at the Bagley locality seemed to be directly attributable to the activities of man. In years past a highway was constructed across the brow of a low hill and then for several hundred yards directly across a low, wet meadow. In building the highway, the roadbed was raised above the surrounding meadow for purposes of drainage. This resulted in the low embankment of the highway being somewhat drier than the adjacent meadow and this has been accentuated by the deepening of the drainage ditches along the sides of the road. In the meadow, especially on one side of the highway, conditions seem to be optimum for *C. muticum*, and this species may have grown there for many years prior to the construction of the highway. *C. discolor*, found most abundantly on the slightly higher ground along the bank of the highway and at the borders of the meadow may or may not be native in this area. There seem to be little doubt, however, that after the construction of the highway the species spread along its margins into the heart of the meadow, and has in this way come into direct association with *C. muticum*. The picture is somewhat complicated by the occurrence of slightly elevated strips in the meadow which are now occupied to some extent by both species, but these in no way obscure the basic features of their relationship. Putative hybrid plants occurred at almost every point where the two species were growing in immediate proximity, but were most abundant along the margin of the highway. There appeared to be in some instances a definite contact zone between the two species where essentially all of the putative hybrids occurred (fig. 1).

Procedures and observations. In order to make statistical comparisons between the two species and their putative hybrids, population samples of

all three were taken. This consisted of making specimens of the inflorescences and middle cauline leaves together with roots where it seemed desirable. The sample of *C. discolor*, taken from twenty-five plants in all, was random in-so-far as it was possible to make it due to the wide dispersal of plants around the periphery of two large colonies of *C. muticum*. The sample of *C. muticum*, also consisting of specimens from twenty-five plants, was taken at random from the center of one colony in an area where *C. discolor* did not

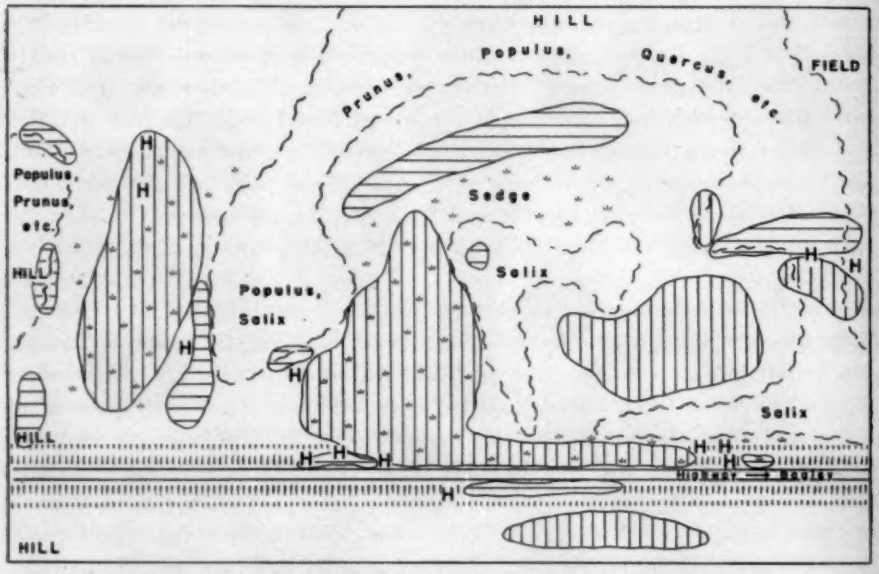


FIG. 1. Sketch of the area where natural hybridization between *Cirsium discolor* and *C. muticum* was found, showing areal relationships between the two species and putative hybrids. Vertical lines, *C. muticum;* horizontal lines, *C. discolor.* "H" marks the points at which one or more hybrids grew. No attempt is made to indicate density of the plants. Willow thickets and woodland are bounded by broken, wavy lines. Distances between features are approximate, the total area sketched being about 8 acres.

occur. Due to the low numbers of the hybrids it was not possible to take a random sample and still secure a number of plants adequate for analysis. Consequently all plants in a large area along and adjacent to the highway which appeared to be of hybrid origin were collected. It was not difficult to recognize putative hybrids in the field because of the conspicuous intermediacy in corolla color, in length of the involucral spines, and pubescence of the under surfaces of the leaves. Thirty plants were collected in whole or in part.

Each plant from which a specimen was taken was scored in the field for the following characters: flower color, diameter of involucre when in full flower and diameter of the stem at the base. Characters later scored in the

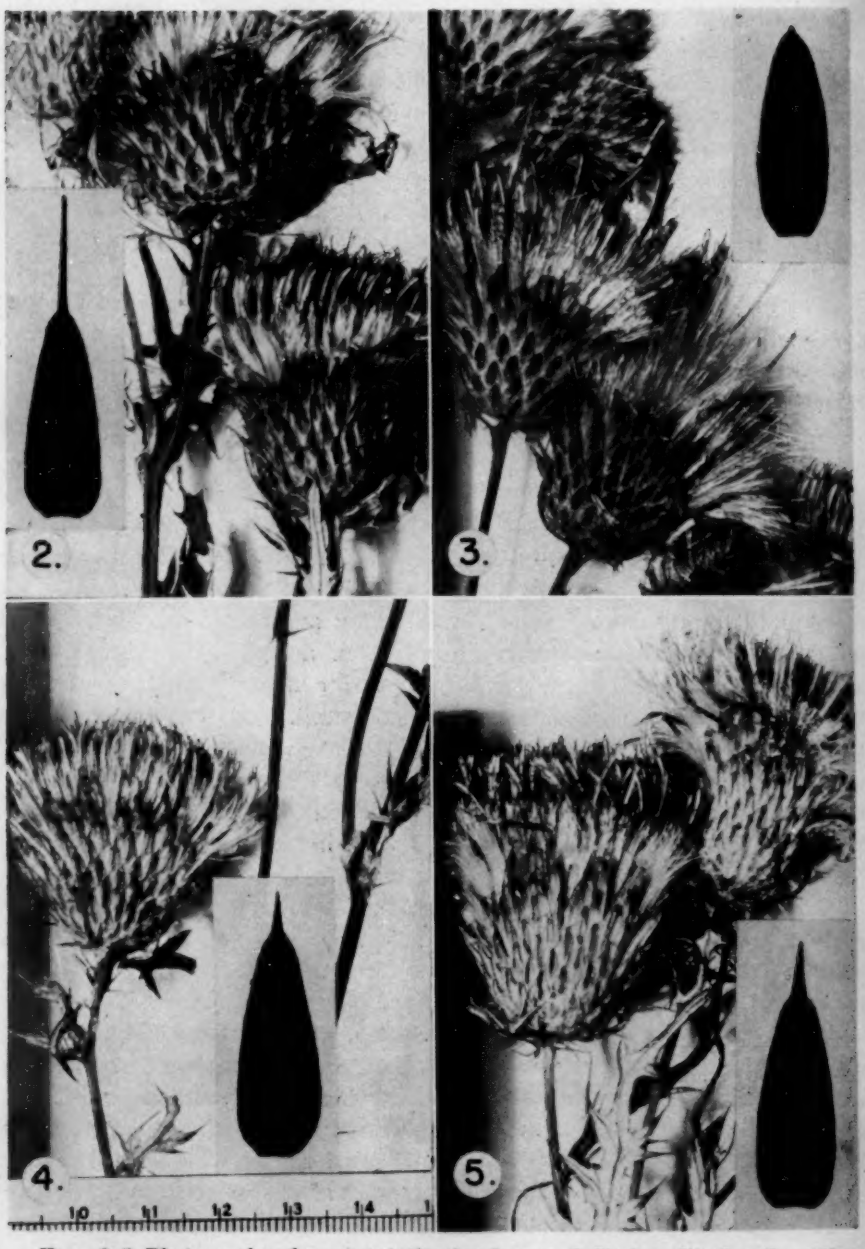
laboratory from the dried specimens were length of the involucral spine, achene color and achene length. Other factors of importance, but which were not scored were found in the nature of the root system, morphology and pubescence of the leaves, achene shape, pollen size, percentage of pollen reaching maturity, and fertility.

In order to present the data collected for the individual plants in the radiate figures (fig. 18), it was necessary to reduce each of the three characters to be used for thus purpose to a common scale as described below.

Scoring of flower color. The values for flower color were assigned in the field according to the following procedure: the deepest lavender of *C. muticum* was given a value of zero, the lightest lavender of *C. discolor* a value of eight. A head with corollas colored as nearly as could be estimated exactly intermediate between the extremes was then selected. All colors of flowers were thenceforth assigned values by direct comparison with these three standard color values as reference points. This method although subjective to some extent was of sufficient reliability to permit assigning of color values to all putative hybrid derivatives with a considerable degree of confidence.

Scoring of length of involucral spines. To insure comparable measurements, the third series of involucral bracts from the base of the head was selected for spine measurements of all three population samples. Several measurements were taken upon each individual and then the average length was calculated. Total range of spine length for all individuals studied was then reduced from absolute measurements in millimeters to a scale of zero to eight. Values for spine length were then determined on the basis of this scale for each plant studied. Due to the very short involucral spine in *C. muticum*, however, the value for spine length in this species appears as zero in every plant. Photographs of representative involucres of both species and the putative hybrids are shown in figures 2–5.

Scoring of achene color. The achenes in *C. muticum* were typically black or deep brown except at the summit where they were pale yellow. In *C. discolor*, the achenes were typically straw colored throughout, occasionally with a few longitudinal streaks of dark brown. Putative hybrids had achenes which ordinarily were of an intermediate color, but an occasional achene was colored as in one or the other parent. The following method of scoring the achenes for color was set up: black or nearly black except at the crown, zero; dark brown, two; brown, the color under magnification seen to be due to numerous longitudinal brownish streaks, the base color being straw, four; straw colored, with a few longitudinal streaks of dark brown six; uniformly straw colored, eight. In practice, a single achene was selected as a standard for the five color values, then by direct matching of other achenes with this constant scale an accurate value could be assigned without difficulty to each achene to be scored.



FIGS. 2–5. Photographs of representative involucres of *Cirsium discolor*, *C. muticum* and their putative hybrids showing variation in spine length (somewhat reduced). FIG. 2. *C. discolor*. FIG. 3. *C. muticum*. FIG. 4. A putative F_1 hybrid between the two species. FIG. 5. A putative back-cross to *C. discolor*. An enlarged silhouette of an involucral bract from near the middle of the head is superimposed in each figure. Scale in millimeters.

Scoring of involucral diameter. Preliminary field observations indicated that the diameter of the involucres when in full flower was generally greater in *C. discolor* than in *C. muticum*. The diameters of several involucres in full flower were, therefore, taken in the field for each plant analyzed, and then from these measurements the average involucral diameter for each plant was calculated. Although there was wide overlap between the two species, the greater average diameter in *C. discolor* was confirmed. The average diameter of the involucres of the putative hybrids was intermediate between the two

TABLE 1. *Tabulation of characters for the individual plants in a population sample of Cirsium discolor. Where characters are assigned a value other than the actual measurements, the range is 0–8.*

Plant no.	Flower color: value	Average spine length: mm.	Average spine length: value	Achene color: value	Achene length: mm.	Average diameter of involucre: mm.	Basal diameter of stem: mm.
1	8	4.0	6	8	4.6	14	9
2	8	5.0	7	–	–	16	12
3	8	5.5	8	7	4.0	14	10
4	8	6.0	8	8	4.6	16.5	24
5	8	5.5	8	6	4.5	16	18
6	8	4.9	7	8	4.2	14.5	22
7	8	5.0	7	8	4.7	15	10
8	8	5.6	8	6	4.1	–	11
9	8	5.4	8	7	4.3	14	17
10	8	6.0	8	6	4.7	15	28
11	8	4.8	7	8	4.5	17	12
12	8	5.2	7	7	4.7	17	10
13	8	6.0	8	5	4.8	15	25
14	8	4.8	7	8	5.2	18	20
15	8	5.5	8	6	4.2	14.5	14
16	8	5.0	7	4	4.5	15.5	20
17	8	5.2	7	7	4.5	15	18
18	8	5.0	7	5	4.4	15	10
19	8	5.0	7	7	5.0	16	35
20	6	4.5	6	5	4.4	16	22
21	8	4.8	7	6	4.1	15.5	7
22	8	5.0	7	4	4.6	–	10
23	8	6.0	8	8	4.8	15.5	13
24	8	5.0	7	7	4.5	14.5	12
25	8	4.0	6	7	4.7	19	20

species. Since this character was to be used on one of the coordinates in the scatter diagrams, figure 18, it was not necessary to reduce these measurements in millimeters to another scale.

Scoring of achene length. Examination of the achenes of both *C. discolor* and *C. muticum* indicated that the average length in the former was greater. Average achene lengths for each plant analyzed were therefore calculated. All measurements were made under magnification to tenths of a millimeter. As with involucral diameters, this character was used on one coordinate of

the scatter diagrams, figure 18. The average achene length in millimeters for each plant was immediately usable for this purpose.

The results of the scoring of the above characters as well as for basal diameter of the stem are shown in tables 1, 2, and 3.

TABLE 2. *Tabulation of characters for the individual plants in a population sample of Cirsium muticum. Where characters are assigned a value other than the actual measurements, the range is 0–8.*

Plant no.	Flower color: value	Average spine length: mm.	Average spine length: value	Achene color: value	Achene length: mm.	Average diameter of involucre: mm.	Basal diameter of stem: mm.
1	0	0.1	0	1	4.3	13.5	15
2	0	0.1	0	1	4.4	12.5	12
3	0	0.1	0	1	4.3	12	7
4	0	0.2	0	–	–	14.5	8
5	0	0.1	0	0	4.5	13.5	20
6	1	0.1	0	1	3.6	12	18
7	0	0.2	0	4	4.4	15	12
8	0	0.1	0	2	4.0	13.5	11
9	0	0.1	0	1	4.0	13	12
10	0	0.1	0	0	4.0	12.5	15
11	0	0.1	0	4	3.7	13.5	18
12	0	0.1	0	0	3.9	12	10
13	0	0.1	0	2	4.2	12	14
14	0	0.1	0	0	3.6	11.5	14
15	0	0.1	0	2	3.4	13	12
16	0	0.2	0	1	3.6	11	10
17	0	0.1	0	2	3.9	12	10
18	0	0.1	0	0	3.6	15	8
19	0	0.2	0	1	3.6	12	15
20	0	0.1	0	1	4.5	14.5	12
21	0	0.1	0	2	3.6	12	10
22	0	0.1	0	–	–	15.5	14
23	0	0.1	0	2	4.2	12.5	24
24	0	0.1	0	0	3.8	12	15
25	0	0.1	0	0	4.5	13.5	9

Stem diameter. Since many of the putative hybrid plants appeared to have been cropped or mowed early in the season, it was evident that height of plant and number of branches could not be used as a measure of gross size. A more reliable index seemed to be stem diameter at the base of the plant, and in all instances this measurement was taken. The overlap in size between plants of the two species according to this index was very great, but the mean diameter of *C. discolor* was greater than for *C. muticum*. The mean diameter for the hybrids was intermediate between the two species.

Leaf morphology and pubescence. In order to make possible a comparison of leaf morphology and pubescence in the two species and their putative hybrids, one to three middle cauline leaves were collected from each plant from which the population samples were taken. An effort was made to collect comparable leaves in each case, viz. those which were fully grown and

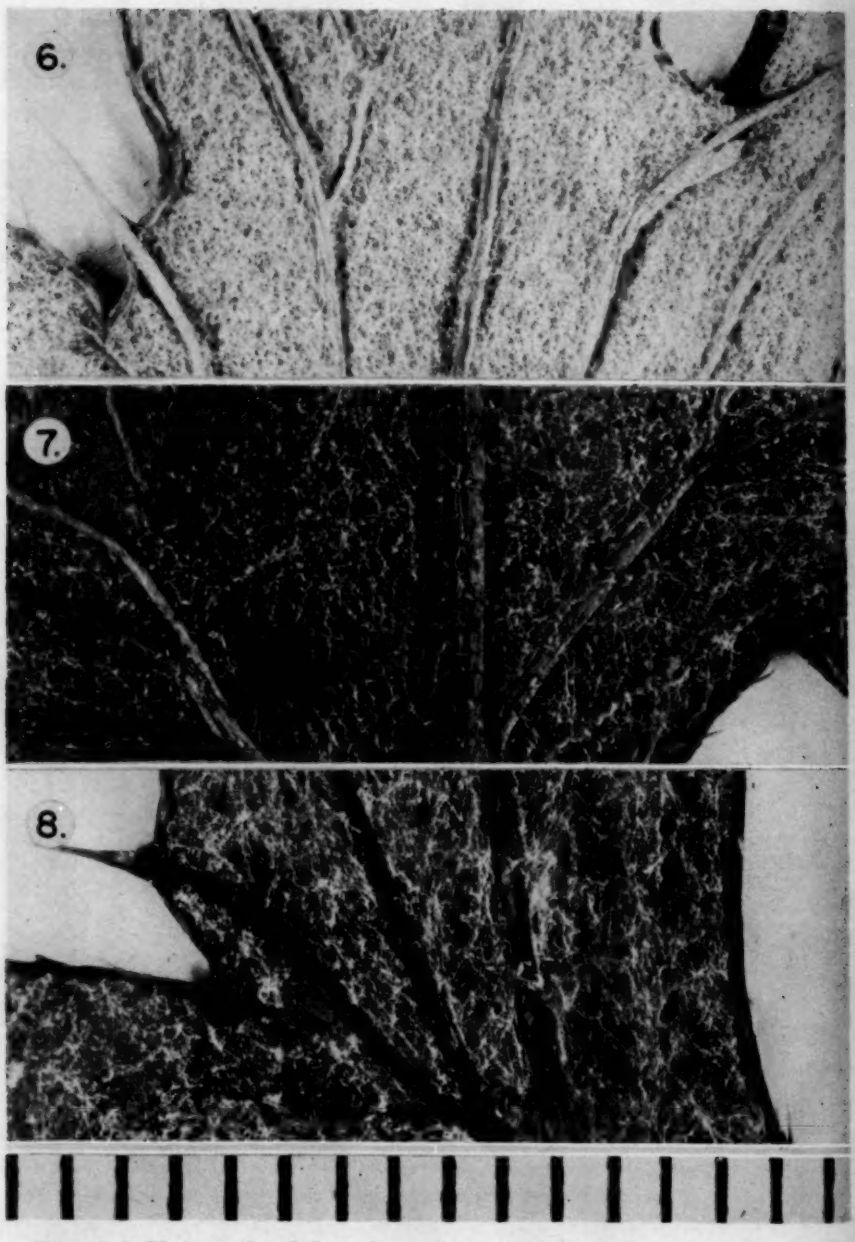
normal in appearance. In applying this method, one objectionable feature was encountered, since plants growing under more favorable circumstances usually were larger and the leaves better developed. Although this factor did not seem to affect outline, it did appear to be directly related to density

TABLE 3. *Tabulation of characters for the individual plants in a population sample of putative hybrids between Cirsium discolor and C. muticum. Where characters are assigned a value other than the actual measurements, the range is 0–8.*

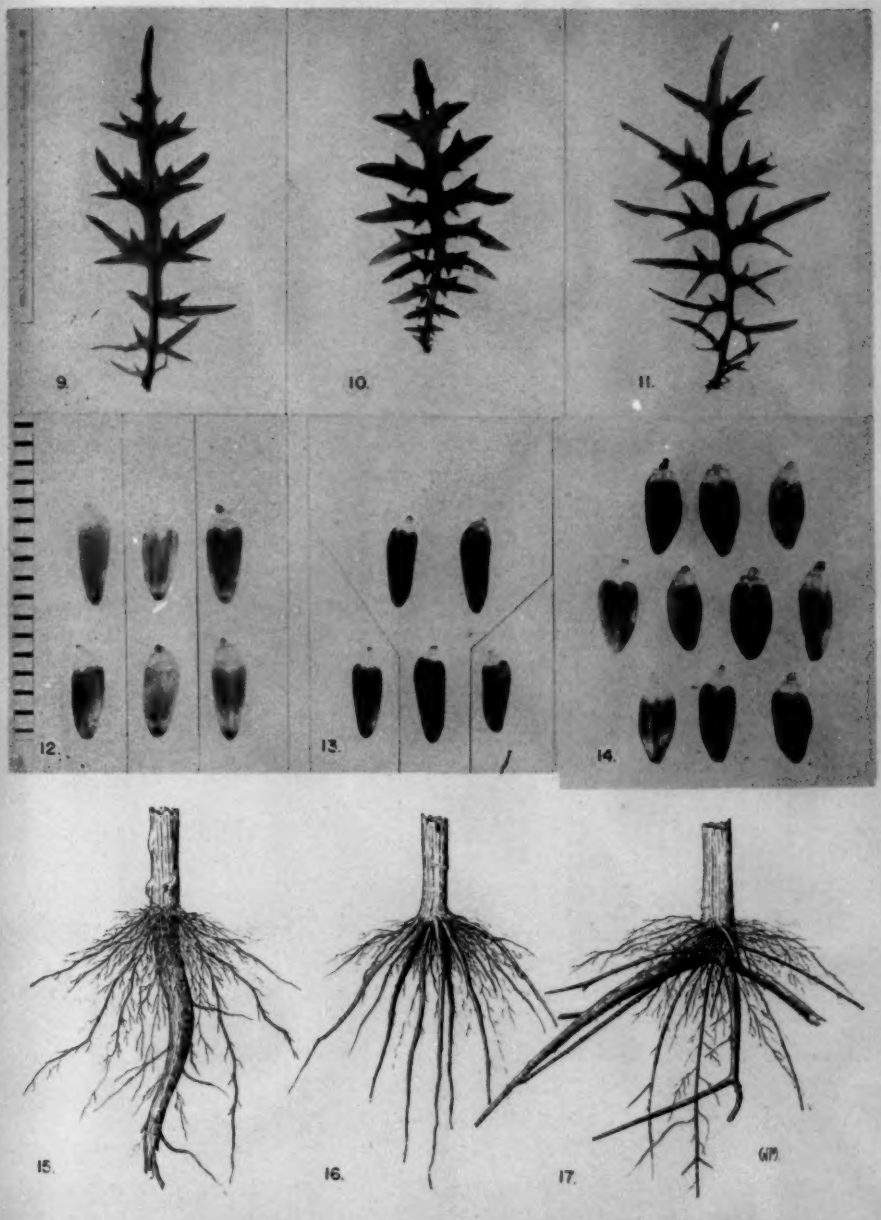
Plant no.	Flower color: value	Average spine length: mm.	Average spine length: value	Achene color: value	Achene length: mm.	Average diameter of involucre: mm.	Basal diameter of stem: mm.
1	8	1.4	2	0	4.3	14	17
2	6	2.0	3	8	5.0	15.5	18
3	2	1.3	1	2	4.3	14	19
4	6	2.4	3	5	4.2	15	6
5	6	1.2	1	4	4.0	16	15
6	4	1.1	1	2	3.8	14	18
7	4	1.6	2	0	4.2	14.5	14
8	8	2.8	4	4	4.6	13.5	12
9	4	1.6	2	4	4.0	15	12
10	2	0.7	1	0	4.5	14	8
11	4	1.6	2	7	4.0	14.5	19
12	4	1.6	2	2	4.0	13.5	15
13	4	1.4	2	2	4.3	14.5	17
14	4	1.5	2	2	4.2	16.5	11
15	–	1.5	2	0	4.0	14	10
16	4	0.8	1	2	4.2	16.5	18
17	4	0.6	1–	0	4.6	16	15
18	4	0.7	1	–	–	15	8
19	6	0.5	1–	8	4.0	14.5	14
20	6	0.6	1–	2	4.6	14.5	20
21	4	1.3	1	2	4.2	14.5	30
22	0	1.8	2	0	4.5	16	15
23	6	1.0	1	2	4.4	16.5	24
24	6	0.9	1	1	4.5	14.5	11
25	6	0.9	1	2	4.0	15	10
26	2	0.3	0	1	4.0	13.5	14
27	2	1.0	1	1	4.0	13	20
28	4	1.3	1	2	4.2	14	10
29	4	0.9	1	3	4.5	13	20
30	4	1.5	2	2	4.4	13	10

of the pubescence since in larger leaves pubescence density per unit area was less, although not enough so to invalidate pubescence-density observations when taken with this factor in mind.

Analysis of these leaf samples revealed several facts of importance. The under surfaces of the leaves of *C. discolor* were always densely tomentose and canescent, those of *C. muticum* usually tomentulose and tending to be green. There were, however, no apparent differences in the nature of this primary pubescence. In both species, especially on the veins of the lower surface and on the upper surface was found a secondary type of pubescence consisting of scattered, translucent, collapsible hairs. These were in no way



FIGS. 6–8. Photographs of the under surfaces of middle cauline leaves of representative specimens. FIG. 6. *Cirsium discolor*. FIG. 7. *C. muticum*. FIG. 8. A putative F_1 hybrid.



FIGS. 9–17. Photographs of representative middle cauline leaves, mature achenes and diagrammatic sketches of root systems of *Cirsium discolor*, *C. muticum* and putative hybrids. FIGS. 9–11 illustrate differences in the lobing of the main leaf segments. FIG. 9. *C. discolor*. FIG. 10. *C. muticum*. FIG. 11. A hybrid. FIGS. 12–14 illustrate differences in shapes of the achenes. FIG. 12. One achene from six different plants of *C. discolor*. FIG. 13. One achene from five different plants of *C. muticum*. FIG. 14. One achene from ten different interspecific hybrids. FIGS. 15–17 illustrate the basic differences between the mature root systems of the three. FIG. 15. Root system of *C. discolor*. FIG. 16. Root system of *C. muticum*. FIG. 17. Root system of a hybrid. All scales in millimeters.

distinctive either in abundance or morphology, and will not be discussed further here. A majority of the hybrids were intermediate in pubescence density (figs. 6–8), but a few plants studied approached the condition found in each parent.

Another more subtle difference between the leaf collections of the two species from this locality was to be found in the nature of the lobing of the middle cauline leaves (figs. 9–10). In *C. discolor* each major segment, especially at the base of the blade, was divided into three lesser lobes, the central one often largest, the lower one nearly or sometimes fully as large or larger. The upper lobe was considerably smaller and was nearer the base of the main segment. In *C. muticum*, the main basal segments of each leaf were again quite distinctly divided into three lesser lobes, but in this case the central lobe was much larger than either of the other two. In contrast to the situation described in *C. discolor*, it was the upper of the two reduced secondary lobes which was the larger, and the lower, smaller one was nearer the base of the main segment. The pattern of lobing described above is not so apparent in the reduced upper cauline leaves.

Examination of herbarium material indicated that the lobing of the middle cauline leaves of *C. discolor* described above is of general occurrence in the species, but in *C. muticum*, throughout its geographical range, the lobing is sufficiently variable to make a similar generalization for this species unwarranted.

In the hybrids, considerably more variability in lobing of the basal segments was found. Each main basal segment had a stout central secondary lobe, but there was a tendency for the other two secondary lobes to be more equal in size and they were often nearly opposed (fig. 11).

Fertility. The percentage of flowers in each head which matured achenes was high in both *C. discolor* and *C. muticum*. In the putative hybrids usually no more than 5% of the flowers set achenes which reached maturity, e.g., the plants were over 95% sterile. Two plants examined which were thought to be hybrids matured above 50% of their achenes (indicated by arrows, (fig. 18). These were interpreted as probable back-crosses to *C. discolor* on the basis of other characteristics.

Achene shape. Besides a conspicuous difference in length of the achenes of the species and the intermediacy of the achenes of the hybrids, there was a less apparent difference in shape. Both of the parental species had achenes which ordinarily were widest near the summit and tapering gradually to the base. The widest point was approximately one-sixth of the distance from the summit to the base in a majority of achenes examined. In the putative hybrids the greatest width was more often about midway between the summit and the base of the achene, or at least was seldom so near the summit

as in the two species. Photographs of achenes showing variation in shape in all three population samples are reproduced in figures 12–14.

Pollen maturation. In order to ascertain the degree of pollen abortion in the species and in the hybrids, aceto-carmine smears were made of mature anthers which had been killed and fixed in a glacial acetic acid-absolute alcohol mixture. When prepared in this way the fully developed pollen grains took the stain readily, the abortive pollen grains remained unstained or nearly so, and translucent. The abortive grains usually were appreciably smaller, and this feature provided an additional check on the accuracy of the method. This method provided no data on the potential viability of the fully formed grains, but it was a rapid and useful way of checking for degree of abortion during their ontogeny.

In all preparations examined, both parental species had a high percentage of fully developed pollen, the highest found in *C. muticum* being 97.8%, the lowest in this species being 90.0%. The highest found in *C. discolor* was 99.2%, the lowest 95.2%. In the putative hybrids, the highest percentage of what appeared to be fully formed pollen seen was 3.0%, and the lowest 1.7%. From these data it is apparent that the expected high percentage of fully formed pollen was present in both *C. discolor* and *C. muticum,* while in the hybrid, as is often the case, the percentage of pollen which reached maturity was very small. The significance of these facts as related to the amount of backcrossing between hybrids and either parent is evident.

A study of the gross size and morphology of the fully developed pollen grains of the two species was made to determine if significant differences could be found. Their pollen appeared to be morphologically identical. The grains were spheroidal and the exine echinate, the large, acute spines being about five microns long. They were of the tricolpate type, the grooves being well developed and conspicuous, each having a large circular pore at the middle. Fully formed grains of the putative hybrids were identical to those of the putative parents in appearance.

In *C. discolor* the average diameter of the expanded pollen including the spines varied considerably in the preparations examined from a minimum of 46.5 to a maximum of 58.0 microns. The average diameter was 51.8 microns. In *C. muticum* the mature pollen varied from a minimum diameter of 49.8 microns to a maximum of 58.0 microns, the average being 54.0 microns. The fully formed pollen of the putative hybrids was so sparse that completely reliable figures could not be secured, but on the whole they appeared to be slightly larger than in either parent, the average diameter of all grains measured being 55.8 microns.

Root system. A rather well marked difference between *C. discolor* and *C. muticum* is found in the root systems. *C. discolor* has a typical deep, stout, persistent tap root (fig. 15). The tap root in *C. muticum* is ephemeral and

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is replaced entirely in the second year by approximately five to eight shallow, well developed laterals (fig. 16). The situation in the hybrids, is intermediate, there usually being about two to four stout main roots which might with equal accuracy be adjudged lateral or tap (fig. 17).

The over all population sample differences are summarized in table 4, and, in part, are expressed pictorially in figure 18.

TABLE 4. *Summary of characters tabulated in population samples of Cirsium discolor, C. muticum and putative hybrids between these species. For further details see Tables 1, 2 and 3, and text.*

Character:	*C. discolor*	*C. muticum*	*Putative hybrids*
Flower color.	pale lavender.	deep lavender.	intermediate, rarely approaching either parent.
Length of involucral spines from third series of bracts.	4.0–6.0 mm.; median 5.0; average 5.1.	0.1–0.2 mm.; median 0.1; average 0.1.	0.3–2.8 mm.; median 1.3; average 1.3.
Color of achenes.	straw, rarely light brown.	black or deep brown, rarely light brown.	straw to black, mostly brown.
Length of achines.	4.0–5.2 mm.; median 4.5; average 4.5.	3.4–4.5 mm.; median 4.0; average 4.0.	3.8–5.0 mm.; median 4.2; average 4.3.
Point of greatest width of achenes.	usually about 1/6 of the distance from summit towards the base.	usually about 1/6 the distance from summit towards the base.	usually about ½ of the distance from summit towards the base.
Diameter of involucre in full flower.	13–20 mm.; median 15.5; average 15.6.	11–16 mm.; median 12.5; average 13.0.	13–17 mm.; median 14.5; average 14.6.
Diameter of stem at base.	7–35 mm.; median 14.0; average 16.4.	7–24 mm.; median 12.0; average 13.0.	6–30 mm.; median 15.0; average 15.0.
Root system.	strong tap.	several stout laterals, no tap beyond seedling stage.	intermediate, sometimes weak tap and one or more secondaries, sometimes 2–4 stout secondaries and no tap.
Pubescence of lower surface of leaves.	densely tomentose.	usually tomentulose.	usually intermediate in pubescence density.
Fertility of flowers.	highly fertile.	highly fertile.	usually less than 5% fertile.
Pollen maturation.	above 95% in flowers examined.	above 90% in flowers examined.	3% or less in flowers examined.

Discussion. The special usefulness of radiate figures combined with scatter diagram techniques (a type of Pictorialized Scatter Diagram; see Anderson, 1949) lies in the opportunity for more characters to be studied simultaneously. In figure 18, for example, it is possible to study the expression and interaction of five characters simultaneously for all individuals analyzed.

Where one or both parental species are unknown, they may also be of use in detecting introgression (see Anderson, l. c.).

In the pictorialized scatter diagrams, figure 18, the diameter of the involucre is plotted on the abscissa, length of achene is plotted on the ordinate. Each population sample has been plotted separately, but the diagrams are arranged on one plate for ready comparison. A key to all characters is given at the upper left corner.

It should be noted that the characters used in the radiate figures, e.g., flower color, length of the involucral spines and achene color were scored as follows: flower color, 0–8, length of spine, 0–8 and achene color, 0–8, *C. muticum* being always at the lower end of the scale, *C. discolor* at the upper end. Then, in accordance with the values assigned, the radiate figures for each plant were drawn so that the length of each radius would be proportionate to the expression of the character.

A further examination of figure 18 shows that there is overlap in the ranges in variability between the species for both involucral diameter and achene length, but the centers of the scatter groups are well separated. The center for the putative hybrids is almost exactly intermediate. In the radiate figures of all three diagrams the radius for achene color shows the highest degree of variability. The putative hybrids display greater over all variability than do either species. Part of this variability can hardly be interpreted as other than resultant from selfing or back-crossing. Two of these plants which apparently were back-crosses to *C. discolor* (they were also much more fertile than the other putative hybrids) are marked on the hybrid diagram with arrows. Still another possible back-cross to *C. discolor* is marked similarly in the *C. discolor* diagram. Since both probable parents of these hybrids were immediately recognized, the usefulness of pictorialized scatter diagrams in detecting introgression is less apparent. If, however, any of the anomalous putative hybrids had been found first in a population of either species when the other species was not in the immediate vicinity, then this in itself would have been of sufficient importance to warrant further investigation.

The bearing of long-continued back-crossing of hybrids to one or both parents upon speciation or upon the origin of units below the species is incompletely understood. The end results of such introgression may be so subtle as to be essentially undemonstrable by present techniques, so much so that no one has yet felt secure in assigning any positive value to the process in evolution (Anderson, 1949; Heiser, 1949). Many instances of introgression may be overlooked because their results are interpreted as normal intraspecific variability.

Most of the plants of *C. discolor* and *C. muticum* illustrated in figure 18 show no evidence of introgression from one to the other species in the

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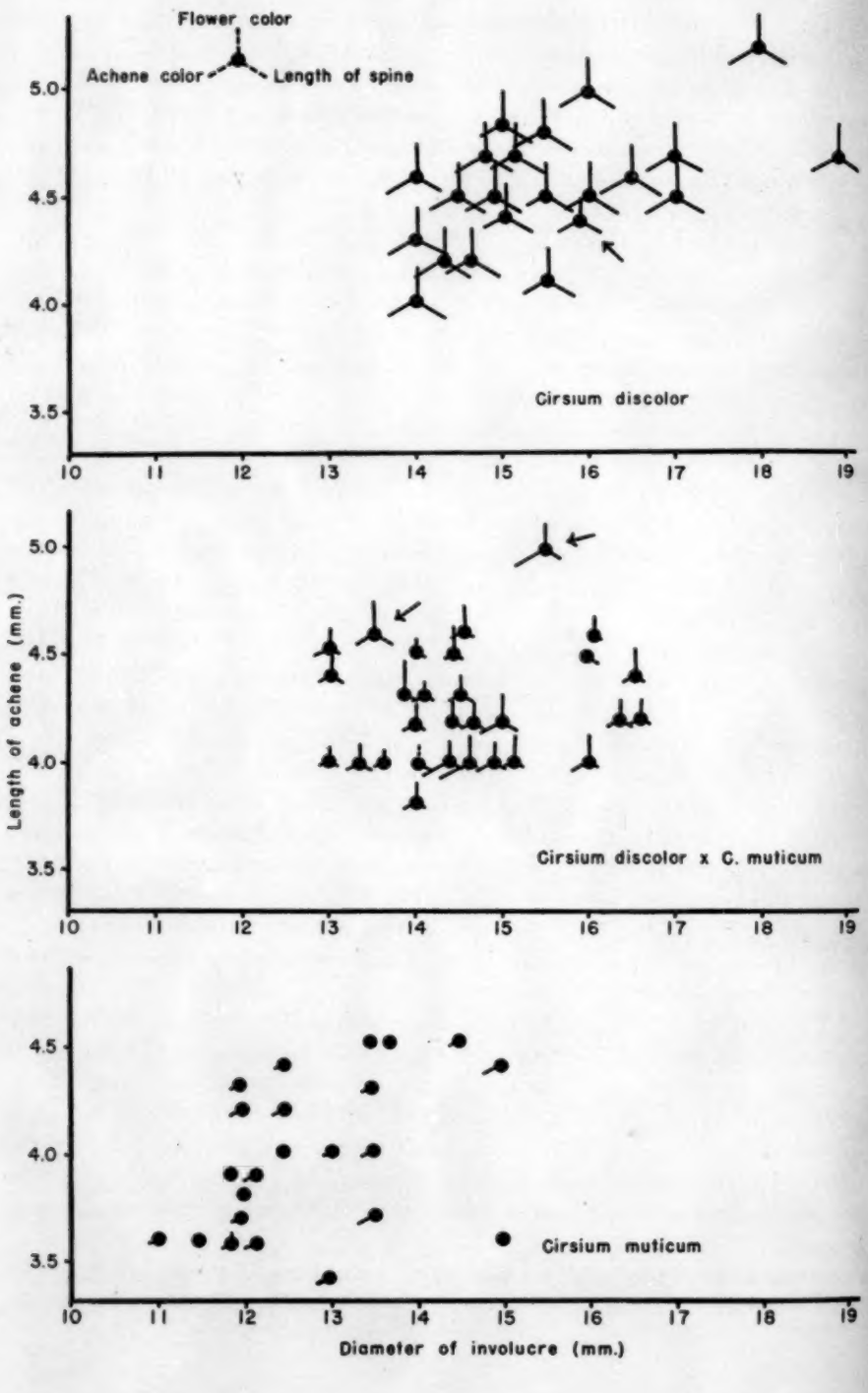
Flower color
Achene color
Length of spine
Cirsium discolor
Cirsium discolor x C. muticum
Cirsium muticum
Length of achene (mm.)
Diameter of involucre (mm.)
5.0
4.5
4.0
3.5
10
11
12
13
14
15
16
17
18
19

characters examined. Some of the figures do, however, provide some evidence of gradual infiltration of germ plasm from *C. discolor* into *C. muticum*. Where this infiltration was pronounced, it is apparent that these plants were immediately construed to be of hybrid origin. In the cases where plants were less evidently intermediate, they were placed almost without hesitation in one or the other species. Most of the plants analyzed in the hybrid diagrams were doubtless first or second generation hybrids, and evolutionarily represent nothing more than possible "bridges" over which traits from one species might pass over to the other. Evidence for introgression from *C. muticum* into *C. discolor* is less apparent although three plants analyzed feasibly could be interpreted as backcrosses to *C. discolor*.

Biologically, it is probably the almost or completely undiscernible instances of introgression which are of the most importance. Variability introduced into a species through introgression manifestly could provide the necessary background upon which selection in the direction of greater adaptability to changing conditions could operate.

Ordinarily the hybrids between two species, other than those which are very closely related, are highly sterile. The flowers of the putative hybrids herein described rarely produced more than 5% mature achenes. Germination tests on achenes from these plants have indicated, however, that the few achenes produced are reasonably viable, but an exact estimate of viability percentage cannot now be made. Furthermore, as discussed above, the hybrids showed a very high percentage of abortive pollen.

It is apparent that both of the above factors, viz. low seed production and high pollen sterility would have an adverse effect on the amount of back-crossing between the hybrids and either parent. One can safely postulate in this instance that the amount of back-crossing is not great. The expected increase in fertility in the back-crosses would, if present, provide sufficient opportunity for germ plasm from one species to be incorporated into the other.

There has been considerable literature which has tended to substantiate the hypothesis that hybrid derivatives are likely to be physiologically intermediate between the parental species in their habitat requirements. Anderson (1948, 1949) believes that the survival of hybrid derivatives in nature is directly attributable to the presence and availability of habitats intermediate between those most suitable to the parental species. If such intermediate habitats are not available, according to him, then there is less

FIG. 18. Pictorialized scatter diagrams of population samples of *Cirsium discolor*, *C. muticum* and putative interspecific hybrids. Length of achene is plotted against diameter of involucre in full flower. In the individual radiate figures, length of the radius is proportional to the expression of the character in that plant. The character expressed by each of the three radii is shown in the figure at the upper left. Arrows indicate putative back-crosses to *C. discolor*. Further explanation and discussion in the text.

likelihood that the hybrid derivative will survive since it is physiologically less suited than non-hybrid individuals of either species for survival in either of their parental habitats. Stebbins, Matzke, and Epling (1947) suggest that there may be ecological selection against hybrid types of *Quercus marilandica* and *Q. ilicifolia* because of the unavailability of such suitable ecological niches.

In the occurrence of the putative hybrids reported in this paper there is nothing which is at variance with the above hypothesis. The preponderance of their occurrence in an intermediate zone between *C. discolor* and *C. muticum* indicates that they are really physiologically intermediate. The probability that the parents were brought together through disturbing and modifying of their previous ecological and geographical relationships by man leads to further inferences. Plant migration progresses most rapidly when the plant communities are open (e.g. the associated species are not in equilibrium) and the opportunities for the seedlings of invading species to become established are at their greatest. Once a plant community has reached a constituency which is relatively stable within the limits of the prevailing ecological conditions there is much less possibility of migration of "foreign" species into it. The invasion of many species into a disturbed area is much facilitated, probably due to greater opportunity for establishment of their propagules. It is at such times that species which potentially are capable of hybridization often are brought together. The presence of a series of ecological niches in the disturbed area which are intermediate between those optimally occupied by these interfertile species would seem to favor fortuitous survival of physiologically intermediate hybrids. Epling (1947) postulates the elimination of the hybrid elements by the restoration of the community to its previous condition of equilibrium through succession. Among several reasons listed by Epling for this result are that the hybrid derivatives would have less chance of survival in competition with parental types already selected for a special environment.

If the forces which result in disturbed conditions remain active, then hybridization may proceed indefinitely even to the point where the original nature of the contributing stocks is difficult or impossible to ascertain. It may be suggested that examples of this type are probably to be sought for most successfully among some of our weedy plants.

Experimental evidence of hybridity. Seeds from a single putative F_1 hybrid when planted germinated poorly. Five seedlings obtained from this planting were grown together with seedlings of both parental species. During one years growth none of these plants developed beyond the rosette stage which is normal in the biennial species of *Cirsium*. Since flowering specimens have not yet been seen, the hybrids could not be checked for segregation of characters in floral and fruiting characters. Segregation

in foliage characters of the first-year plants was, however, conspicuous. One of the five hybrids showed a preponderance of characters in the direction of *C. muticum*. The other four hybrid plants varied in the direction of *C. discolor*. All five hybrids showed a marked loss in vitality as compared to the plants of the parental species. One hybrid was of such low vitality that it may never reach the flowering stage.

A second planting of seed from another putative hybrid resulted in three seedlings. All three plants at the end of one years growth closely resembled *C. muticum* in appearance, but in the secondary lobing of the leaves exhibited hybrid characters. There was no indication of a loss in vitality in these three plants. Quite probably the plant from which the seeds were obtained was itself a first or second generation back-cross to *C. muticum*.

Chromosome numbers. Chromosome counts were made from root tips from seedlings of both the species and the hybrids The hybrid seedlings were at least of the second generation since the seeds for this planting were obtained in the field from a highly sterile putative hybrid. The somatic number of chromosomes for both the species and the hybrid was determined to be 20. Chromosome morphology in *C. discolor* and *C. muticum* was not conspicuously different. However, more detailed studies may ultimately reveal minor differences between thm.

Equal chromosomal complements of the parent species may be of considerable importance in the production of hybrid derivatives beyond the first generation. This permits equal pairing of the chromosomes during meiosis in the hybrids. That the pairing is by no means complete is indicated by the high percentage of pollen abortion and of sterility in the hybrid plants. The fact that backcrossing to either parent possibly is not common is not of greatest significance. Actually, low compatibility of two species may well be of considerable importance in effecting introgression between them. This presumably would prevent swamping of one or both species by the other.

SUMMARY

1. Natural hybridization between species of North American thistles apparently first was postulated by Asa Gray about seventy-five years ago. Subsequent authors, particularly Rydberg and Petrak, continued to report its occurrence without attempting other than descriptive records.

2. This paper constitutes an attempt to analyze through population samples a rather distinctive occurrence at one locality of putative natural hybridization between two species, *C. discolor* and *C. muticum*. These two species appeared to have been brought together through disturbances of their natural environments. For the most part the individual plants seemed

to have maintained fully their specific identities. Numerous interspecific hybrids, occurred, however, in an area which roughly was along the zone of contact between the species.

3. Population samples of the species and their hybrids were collected. Each individual plant was tabulated for flower color, diameter of the involucre, length of involucral spines, color of achenes, length of achenes and basal diameter of the stem. Pictorialized scatter diagrams were then constructed for each plant of all three population samples utilizing all characters tabulated except basal diameter of the stem. A study of these diagrams indicated that most of the hybrids were intermediate between the parents in all of the characters analyzed. There was evidence of some back-crossing to both parents, particularly to *C. muticum*.

4. Analysis of the root systems and pubescence and morphology of the leaves of both species and their hybrids indicated that the latter were also intermediate in their expression of these characters, although their ranges in variability were greater.

5. A study of the pollen demonstrated that the hybrids ordinarily produced less than 3% fully formed pollen. Both species produced over 90% fully formed pollen in all flowers examined.

6. In a great majority of cases, less than 5% of the flowers in the hybrids matured their achenes. In both *C. discolor* and *C. muticum* a large majority of the flowers were fertile and matured achenes.

7. Hybridization at the locality studied although continuing, apparently has not altered materially the parental species through introgression. There is, however, some evidence of introgression of *C. discolor* into *C. muticum*, and less evidence of introgression in the opposite direction. The ultimate products of introgression may, however, be of such a nature that they have been rendered undetectable by the methods used.

8. It seems probable in this instance of natural hybridization that the hybrids are physiologically intermediate between their parents, and that this intermediacy is evidenced by their intermediate habitat preferences.

9. Evidence substantiating the reality of natural hybridization between *C. discolor* and *C. muticum* is supplied by the progeny of a putative F_1. Five first year seedlings from this plant exhibited conspicuous segregation of foliage characters together with a marked loss in vitality of the plants.

10. The diploid number of chromosomes determined from root tips was found to be 20 in *C. discolor*, *C. muticum* and in their hybrids.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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CYTOGEOGRAPHY OF PHYSALIS IN WEST AFRICA

J. T. BALDWIN, JR. AND BERNICE M. SPEESE

As weeds or as cultivated plants three representatives of *Physalis* occur in west tropical Africa: *P. angulata* L., *P. peruviana* L., *P. divaricata* Don. R. D. Meikle of the Royal Botanical Gardens at Kew kindly identified some of our specimens: those of the first two species match the Linnean types; another specimen fits *P. divaricata*. This last entity has a complex nomenclatorial history. Hutchinson and Dalziel (1931) included only *P. angulata* in the *Flora of West Tropical Africa*.

The West African native often grows a number of different kinds of plants intermixed with a major crop; he scatters seeds of those plants in a selected part of the field and apparently pays them little further heed. In time the desired plant parts are utilized. In Liberia, for example, upland rice is by far the most important food-plant in the native economy; the total acreage of this crop for a population of *ca.* 800,000 is considerable. *Zea* is frequently, and *Pennisetum* and *Vigna* occasionally, interplanted with the rice, and one readily recognizes them as cultivated plants. But *Amaranthus, Talinum, Solanum, Physalis, etc.*, are likewise found in the fields or about the dooryards, and often one does not know whether certain individuals are spontaneous or not. *Physalis* in West Africa may be best described as a weed that is in some instances cultivated. Seldom, if ever, is *Physalis* planted in thick stands like one commonly sees for this genus in Mexico and Guatemala.

Incidental to assignments in West Africa for the United States Government Baldwin has accumulated data on *Physalis* and made collections of herbarium specimens and seeds. Speese has grown representative plants at Williamsburg, Virginia, and examined their chromosomes. The various collections are listed here; specimens have been deposited. as indicated, in the herbaria at (B)eltsville, Maryland, at (K)ew, and at the (M)issouri Botanical Garden:

Physalis angulata L.				*n*	2*n*
LIBERIA					
Baldwin	*5875*	: Monrovia	(B)	24	.....
	5934	: Harbel	(K)	.....	.....
	11595	: Nana Kru	(B)	.....	.....
	14529	: Monrovia	(B, K)	. .	48
	14535	: Monrovia	(B, K, M)	24	.....
FRENCH GUINEA					
	9765	: Macenta	(B, K)	.....	.....
NIGERIA					
	14534	: Ikom	(B, K)	.....	48

FRENCH CAMEROONS				
	14530 : Bertoua	(B, K)	.....	48
Physalis divaricata **Don.**				
LIBERIA				
	6433 : Jabroke	(B, K, M)	12	.....
	6755 : Gletown	(B)	.....	.....
GOLD COAST				
	13550 : Tamale	(B)	.....	.....
NIGERIA				
	14531 : Agege	(B, K, M)	.....	24
FRENCH CAMEROONS				
	14533 : Bertoua	(B)	.....	24
Physalis peruviana **L.**				
BRITISH CAMEROONS				
	13853 : north of Bamenda	(B, K, M)	.....	48

The senior author found *P. angulata* to be an occasional weed throughout the areas visited. It was most often in the vicinity of villages, especially in fairly wet situations. On fertile soils plants sometimes exceeded a height of three feet. The flowers are yellow cream, usually with brownish markings inside the corolla; the filaments are brownish and not hairy; the anthers, bluish. Mahogany anthocyanins are present in the calyces and peduncles of some plants. The fruits are *ca.* 1.25 centimeters in diameter and edible; they are greenish yellow, or in some forms, according to Dalziel (1948), red. Other plant parts have reputed medicinal values (Dalziel, 1948). Chromosome numbers are $2n = 48$ (fig. 1), $n = 24$ (fig. 2). Nakajima (1931) reported an n-number of 12 for this species; Yamamoto and Sakai (1932) and Tokunaga (1934), an n-number of 24; Young (1949), a $2n$-number of 48. Tokunaga states that his plants and those investigated by Nakajima were all from Utsunomiya Agricultural College, Japan, where they had been introduced for experimental purposes. It is altogether likely that Nakajima wrongly identified his material.

P. divaricata is occasionally common in fields. But it may also be found in waste places, as among the rocks on Mamba Point, Monrovia, Liberia. The plant is prostrate, or tends to be, with a stem as much as a meter in length. The flowers under greenhouse conditions are cream white, with white, hairy filaments and yellow anthers; the fruit are small and greenish. In the field the flowers were observed to be yellowish, and the fruit greenish purple. Chromosome numbers are $2n = 24$ (fig. 3) and $n = 12$ (fig. 4). Seemingly no chromosome reports for *P. divaricata* are in the literature.

In his extensive travels in West Africa, Baldwin saw only two plants of *P. peruviana:* one of them at Jos, Bauchi Plateau, Nigeria, May 9, 1948; the other, about fifteen miles north of Bamenda, British Cameroons, December 8, 1949 (*Baldwin 13853*). This species is widely cultivated, often being

known as the "Cape Gooseberry" (Ward, 1949), so it is possible that the two plants observed were escapes. But of significance may be the fact that

FIGS. 1–5. Chromosomes of *Physalis*. FIGS. 1–2. *P. angulata:* $2n = 48$, $n = 24$. FIGS. 3–4. *P. divaricata:* $2n = 24$, $n = 12$. FIG. 5. *P. peruviana:* $2n = 48$. As shown in this drawing, certain chromosomes of *Physalis* do not exhibit centromeres after treatment with paradichlorobenzene. Pollen mother cells and leaves were used in making chromosome studies. × 2200.

Jos and Bamenda are disjunct areas characterized by montane vegetation (see vegetation map, Hutchinson and Dalziel, 1936): during slave-trading days, when traffic between the coast and those areas was most likely heavy,

it may be that the species was introduced from South America and has now become established as a weedy element of the montane vegetation. The Bamenda plant was at an elevation of *ca.* 5,000 feet, with *Trifolium, Clematis, Rubus,* etc., as associates. It was growing among other plants to a height of four feet; it was beside a small stream and in a region heavily grazed by cattle. No human habitations were close, though herdsmen were frequently there. The Jos plant, at about 4,200 feet, was on the very outskirts of the city.

P. peruviana near Bamenda has a $2n$-number of 48 chromosomes (fig. 5). For this species de Vilmorin and Simonet (1927) reported that $n = 24$; Yamamoto and Sakai (1932), for plants of commercial origin, that $n = 12$; Bhaduri (1933), for plants from the vicinity of Calcutta, India, that $n = 24$; Young (1949), that $2n = 48$. Again, discrepancies in chromosome reports may reflect erroneous identification.

SUMMARY

1. Three representatives of *Physalis* occur in West Africa: *P. angulata* L. (for five collections, $2n = 48$ or $n = 24$), *P. peruviana* L. (for one collection, $2n = 48$), and *P. divaricata* (for three collections, $2n = 24$ *or* $n = 12$).

2. That similar and different chromosome reports are in the literature for *P. angulata* and *P. peruviana* is probably indicative of faulty taxonomic determination. These three representatives of *Physalis* are usually weeds in West Africa, but in some instances plants may be cultivated.

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SUPPLEMENTARY NOTES ON AMERICAN MENISPERMACEAE—V

B. A. KRUKOFF AND H. N. MOLDENKE

Since our last serial paper on American *Menispermaceae* appeared in print (Krukoff and Moldenke 1947) we have examined approximately 200 new collections. The great majority of these specimens were recently collected by the collectors associated with the Instituto Agronomico do Norte, Belém, Brazil, in connection with their present extensive botanical explorations of the basin of the Amazon.

The collections examined extend our knowledge of certain species, extensions of ranges are noted for a very considerable number of species, one species, *Sciadotenia javariensis* Moldenke, is described as new, and one new combination, *Anomospermum froesii* (Krukoff and Moldenke) Moldenke, is proposed.

The species noted in this supplement are arranged in the same order, and the place of deposit of specimens is shown by the same abbreviations, as in our previous papers (Krukoff and Moldenke 1938, 1939, 1941, 1942, 1943, 1947). The following additional abbreviations are used: Ja: Museo Nacional, Rio de Janeiro. Oa: Botanical Museum, Cambridge, Massachusetts. Ug: Museo de Historia Natural, Montevideo.

Certain specimens from the State of Espirito Santo, Brazil, were sent to us under temporary numbers ("OVB-1", etc.) from the Museo Nacional at Rio de Janeiro. They are deposited in the herbarium of the New York Botanical Garden as are all other specimens hereinafter cited where no other place of deposit is indicated.

CHONDODENDRON RUÍZ & PAVON

1. CHONDODENDRON MICROPHYLLUM (Eichl.) Moldenke. BRAZIL—Bahia: Ilheos de São Pedro, *H. P. Vellozo 931* (Ja).

2. CHONDODENDRON PLATIPHYLLUM (A. St. Hil.) Miers. BRAZIL—Ceará: *Freire Allemão & M. de Cysneiros 33* (Ja). Espirito Santo: basin of Rio Doce, between its affluent Rio Pancas and the town of Linhares, *OVB-1, OVB-31, OVB-34, OVB-36, OVB-73, OVB-84, OVB-85, OVB-86, OVB-93.* Rio de Janeiro: *Mus. Nac. Rio Jan. 6785* (*Souza Brito s.n.*) (Ja), *45410* (*Schreiner s.n.*) (Ja), *45427* (*Pimenta Velloso s.n.*) (Ja), *45428* (*Glaziou s.n.*) (Ja), *45430* (*Coll. undesign. 405*) (Ja), *45431* (*Schwacke s.n.*) (Ja); *A. J. Sampaio 3104* (Ja), *3323* (Ja); *Diogo 685* (Ja); *Brade 9556* (Ja); *Neves Armond 58* (Ja); *A. L. & H. N. Moldenke 19614* (outside of Parque Nac. da Serra dos Orgãos) (Ja, N, Ug).

These are the first records of the species from the States of Ceará and Espirito Santo.

3. CHONDODENDRON TOMENTOSUM Ruíz & Pav. PERU—San Martín: Chazuta, *Klug 3* (N, Ug), *3b, 10, 47, s.n.*

4. CHONDODENDRON CANDICANS (L. C. Rich.) Sandw. BRITISH GUIANA—Essequibo; Keriti Creek, *Forest Dept. 3623.*

5. CHONDODENDRON LIMACHFOLIUM (Diels) Moldenke. COLOMBIA—Amazonas: vicinity of Leticia, *Schultes & Lopez 10400g* (N, Oa). BRAZIL—Amazonas: basin of the upper Jurua, munic. Eirunepe, ? *Fróes 21720,* ? *21736;* basin of Rio Negro, *Fróes 21446* (Be); basin of Rio Solimoes, *Fróes 20938* (Be), *23755B* (Be), *23919* (Igarape Jandiatuba) (Be). Pará: basin of Rio Solimoes, Jauarisinho, *Fróes 23450, 23453.*

These are the first records of the species from Colombia, and from the basins of Rio Negro and Rio Tocantins in Brazil.

7. CHONDODENDRON TOXICOFERUM (Wedd.) Krukoff & Moldenke COLOMBIA—Vaupés: basin of Rio Vaupés, *Schultes 5526* (Rio Macaya). BRAZIL—Amazonas, basin of the upper Jurua, munic. Eirunepe, *Fróes 21728, 21746.*

This is the first record of the species from the basin of Rio Vaupés, Colombia.

The collector states on the label (*Schultes 5526*) "said to have been used formerly by Karijona Indians in arrow-poison."

SCIADOTENIA MIERS

1. SCIADOTENIA SAGOTIANA (Eichl.) Diels. BRAZIL—Amazonas: basin of Rio Negro, *Ducke 1967; Fróes 22855.*

These are the first records of the species from the State of Amazonas.

2. SCIADOTENIA PARAENSIS (Eichl.) Diels BRAZIL—Pará: basin of Rio Tapajos, *Black 74-1880;* basin of Rio Capim, *Schwacke 146* (Ja); basin of Rio Guamá, *Fróes 20449* (Be); near Belém, Black *835* (Be); *Murça Pires & Black 531;* estrada de Ferro de Bragança, *Fróes 20767* (Be).

6. SCIADOTENIA RAMIFLORA Eichl. BRAZIL—Amazonas: *Schwacke 551* (Ja).

This is the first record of the species from Brazil.

8. SCIADOTENIA AMAZONICA Eichl. BRAZIL—Amazonas: basin of Rio Negro, *Fróes 20472* (Be).

This is the second collection of the species from Brazil.

9. SCIADOTENIA CAYENNENSIS Benth. BRAZIL—Pará: basin of Rio Cuminá, *A. J. de Sampaio* (Ja.)

10. SCIADOTENIA BRACHYPODA Diels. BRAZIL—Amazonas: basin of Rio Negro, *Murça Pires 906* (Be); basin of Rio Solimoes, *Fróes 20526* (Be).

This is the first record of the species from the basins of Rio Negro and of Rio Solimoes.

11. SCIADOTENIA SIMILIS Moldenke BRAZIL—Amazonas: basin of Rio Içá, *Fróes 20857* (Be).

Up to the present the species was known only from two collections, one from the basin of Rio Negro in Brazil (type coll.) and another from Leticia, Colombia.

13. SCIADOTENIA TOXIFERA Krukoff & Smith. COLOMBIA—Putumayo: basin of Río Putumayo, between Puerto Ospino and Concepcion, *Schultes 3866*. BRAZIL—Amazonas: basin of Rio Solimoes, Igarape Belém, *Fróes 23755;* basin of the upper Jurua, munic. Eirunepe, Igarape Folguedo, near Assahytuba, on varzea land, *Fróes 21716, 21717, 21719, 21725*.

Up to the present the species was known only from two collections from Ecuador and two collections from Peru (near Iquitos).

According to the collector (*Schultes 3866*) roots and stems are used in Kofám curare and the plant is known under the name "Ya-pé-hĕ".

14. **Sciadotenia javariensis** Moldenke, sp. nov.

Frutex scandens; ramis graciliusculis paulo contortis multistriatis, in statu juvenili dense breviterque brunneo-publescentibus, denique glabrescentibus; ramis brevibus dense brunneo-pubescentibus; petiolis gracilibus ca. 5 cm. longis dense brunneo-pubescentibus genuiflexuis; laminis foliorum suborbicularibus chartaceis utrinque in siccitate brunnescentibus, ca. 7 cm. longis et 8 cm. latis integris, ad apicem rotundis, ad basim rotundatis vel subtruncatis, supra glabratis, subtus strigosis, 5-pli-nerviis non bullatis; inflorescentibus masculis paniculatis ca. 30 cm. longis et 6 cm. latis multibrachiatis; sepalis 18 elliptico-lanceolatis dense strigoso-pubescentibus, petalis 6 obovatis pilosis; staminibus 6 ad basim irregulariter connatis.

Woody vine; stems rather slender, round in cross-section, solid, somewhat twisted, many-striate, densely short-pubescent with dark brown hairs when young, glabrous in age and with the outer layer of bark readily peeling off; pith small, white, 3-lobed; branches apparently short, densely brown-pubescent with short spreading or somewhat appressed hairs; leaf-scars on older wood large, cordate at the apex, with thick projecting margins; petioles slender, about 5 cm. long, densely brown-pubescent, genuiflexuous near the apex, the upper 5–6 mm. firmer, darker, and less pubescent; leaf-blades suborbicular, widest somewhat below the middle, chartaceous, brunnescent on both surfaces in drying, about 7 cm. long and 8 cm. wide, entire, rounded at apex, rounded or subtruncate at base, glabrate above, strigose beneath with short brownish hairs that are slightly more abundant on the larger venation and in general point toward the margins, not bullate above, 5-pli-nerved, the central vein strongest, the 2 lateral ones not quite as prominent, the 2 basal very slender, all anastomosing at the very margin beneath; secondaries and tertiaries rather abundant, irregular, subprominulous beneath; inflorescence paniculate, about 30 cm. long, 6 or more cm. wide, many-branched; peduncles very slender, about 6 cm. long, densely short-pubescent; rachis and its branches similarly very slender or subfiliform and densely brown-pubescent; pedicels filiform, 0.5–1.5 mm. long, densely brown-pubescent; flowers green, only staminate ones seen; sepals 18, elliptic-lanceolate, varying in size, the outermost about 0.8 mm. long and 0.4 mm. wide, the inner ones to 1.8 mm. long and 0.9 mm. wide, attenuate-acute at the apex, concave, slightly fleshy, densely strigose-pubescent on the back; petals 6, rather fleshy, obovate, about 0.8 mm. long and 0.4 mm. wide, rounded at the apex, pilose on the back and margins; stamens 6, erect or variously divergent; filaments fleshy, glabrous, irregularly united near the base, about 0.4 mm. long; anthers 2-celled, dorsifixed.

The type of this species was collected by Adolfo Ducke (*no. 1970*) on low terra firma at Esperanca, at the mouth of Rio Javary, Amazonas, Brazil, on September 28, 1942, and is deposited in the herbarium of the Instituto Agronomico do Norte at Belém. The species is apparently most closely related to *S. eichleriana* Moldenke and *S. solimoesana* Moldenke, from both of which, however, it may be at once distinguished by its suborbicular leaf-blades which are only 5-pli-nerved and are strigose over the whole lower surface. BRAZIL—Amazonas: basin of Rio Javary, *Ducke 1970* (Be-type).

Anomospermum Miers

1. Anomospermum schomburgkii Miers. BRAZIL—Minas Geraes: *Mus. Nac. Rio Jan. 25096* (*Schwacke s.n.*) (Ja, N). Rio de Janeiro: *Mus. Nac. Rio Jan. 45422* (*Glaziou & Schwacke s.n.*) (Ja). Amazonas: basin of Rio Negro, *Fróes 21108* (Be), *22480, 22554; Murça Pires 340.* Pará: basin of Rio Tapajos, *Black 47-1995.*

This is the first record of the species from the State of Minas Geraes.

3. Anomospermum reticulatum (Mart.) Eichl. PANAMA—Coclé: El Valle de Anton, ? *Allen 3538.* BRAZIL—Amazonas: basin of Rio Solimoes, Jandiatuba, *Fróes 23855;* basin of Rio Negro, Manaos, *Ducke 1966* (Be); *Mus. Nac. Rio Jan. 45423* (*Schwacke s.n.*) (Ja). Pará; basin of Rio Amazonas, Tefe, *Murça Pires 1340* (Be); *Fróes 20633;* basin of Rio Tajapurú, *Black 48-2963* (Be).

Local names: Yoira (basin of Rio Tajapurú). Gogo de guariba (Fonte Bôa).

The specimen from Panama is probably a new species related to *A. reticulatum* and which we do not wish to describe at this time because of the absence of good flowering material. Of the eleven known species of *Anomospermum* all are confined to the basin of the Amazon (Brazil, Peru, Colombia, Ecuador and Bolivia) and the adjacent three Guianas, with the exception of the three, namely, *A nitidum* which is found in northeastern Brazil, the recently described *A. occidentale* Cuatrecasas from western Colombia, and the common and widespread *A. schomburgkii* which has been reported from as far north as Trinidad and as far south as Rio de Janeiro.

This is the first record of the species from the basins of Río Solimoes and Río Negro in the State of Amazonas and from the basin of Rio Tajapurú in the State of Pará.

6. Anomospermum chloranthum Diels. BRAZIL—Amazonas: basin of Rio Negro, *Fróes 21466* (Be).

8. Anomospermum bolivianum Krukoff & Moldenke. BRAZIL—Pará: basin of Rio Tocantins, *Fróes 23458.*

This is the first record of the species from the State of Pará.

9. Anomospermum hirsutum Krukoff & Moldenke. BRAZIL—Amazonas: basin of Rio Negro, *Black 48-2473, Fróes 21154, 21440.*

Fróes 21440 is the first pistillate specimen seen by us, but it is already in fruit. Whether the fruits are mature or not is not certain. They are borne on slender fruiting-pedicels 2.3–2.5 cm. long, sparsely hirsute or glabrescent,

nigrescent, about 0.5 mm. in diameter from the base to near the apex, expanded to 2 mm. at the torus; fruit elliptic-asymmetric, small, 0.7–1.2 cm. long, 4–7 mm. wide, very minutely pulverulent-puberulent or glabrate, sometimes subapiculate at the apex, the ectocarp and mesocarp combined about 1 mm. thick, not separating, the endocarp chartaceous smooth, shiny, black.

11. ANOMOSPERMUM GLAUCESCENS Moldenke, Phytologia **3**: 121–122. 1949. BRAZIL—Pará: basin of Rio Tocantins, *Fróes 23446* (N-type, Be-isotype).

12. **Anomospermum froesii** (Krukoff & Moldenke). Moldenke, comb. nov. *Abuta froesii* Krukoff & Moldenke, Bull. Torr. Bot. Club **70**: 404. 1943.

When this species was described by us we had only stems, leaves, and fruit available. Judging from this material we assumed that the species was a member of the genus *Abuta*. Now, however, an unnumbered collection of Dr. Ducke provides us with flowers for the first time. Fortunately the flowers are staminate, for it is on the basis of the presence or absence of petals in the staminate flowers that the genera *Abuta* and *Anomospermum* are distinguished. The flowers prove that our plant is an *Anomospermum*, and the necessary new combination is therefore proposed above.

A description of the staminate flowers follows: sepals 6, the 3 outer ones minute, about 0.5 mm. long and wide, rhombic in shape, acute at the apex, bract-like, the three inner ones very large, broadly elliptic-subrotund, thick-textured, about 3 mm. long and 2.5 mm. wide, rounded at the apex, concave on the inner and convex on the outer surface, glabrous on both surfaces; petals 6, fleshy, oblong, about 2 mm. long and 1 mm. wide, glabrous, the margins folded inwards and partly enfolding the stamens; stamens 6, opposite to and partly enveloped by the petals, free and separate from each other; filaments stout, erect, about 2 mm. long, glabrous; anthers small, yellow, elliptic, about 0.5 mm. long, not wider than the filament, the two thecae lateral, dehiscing by means of a continuous longitudinal slit. BRAZIL—Amazonas: basin of Rio Negro, *Ducke s.n.* (July 25, 1948).

TELITOXICUM MOLDENKE

1. TELITOXICUM KRUKOVII Moldenke. BRAZIL—Amazonas: Comitian, *Fróes 24028* (Be).

2. TELITOXICUM GLAZIOVII Moldenke. BRAZIL—Pará: basin of Rio Tapajos, Santarem, *Jobert & Schwacke* 912 (Ja). Ceará: *Freire Allemão & M. de Cysneiros 28* (Ja).

It is very satisfactory to examine the above-mentioned two collections. The species has been known previously only from a single collection of a cultivated plant from Rio de Janeiro.

6. TELITOXICUM MINUTIFLORUM (Diels) Moldenke. BRAZIL—Amazonas: basin of Rio Solimoes, Igarape Belém, *Fróes 23755c* (Be).

ABUTA BARRÈRE

1. ABUTA MACROCARPA Moldenke. BRAZIL—Amazonas: basin of the upper Jurua, munic. Eirunepe, *Fróes 21735* and *21737* (Igarape Folguedo, terra firma), *21772* (Parana Japura, terra firma); basin of Rio Solimoes,

Fróes 23755A (Igarape Belém), *23758* and *23920* (Igarape Jandiatuba), *24000* (Camatian).

2. ABUTA OBOVATA Diels, BRITISH GUIANA—"Bartica-Potaro road", *Forest Dept. 4233.* BRAZIL—Amazonas: basin of Rio Solimoes, Igarape Belém, *Fróes 23758;* basin of Rio Negro, *Black 48-2632.*

This is the first record of the species from the basin of Rio Solimoes.

3. ABUTA BULLATA Moldenke. BRAZIL—Amazonas: basin of the upper Jurua, munic. Eirunepe, Igarape Folguedo, near Assahytuba, *Fróes 21718, 21726, 21755;* basin of Rio Solimoes, Camatian, *Fróes 23964.* Pará: near Belém, *Barbosa da Silva 709* (Be).

The three cited collections from the upper Jurua were collected on varzea, on restinga alta and on terra firma. This is the first record of the species from the State of Pará.

5. ABUTA VELUTINA Gleason. BRAZIL—Amazonas: basin of Rio Içá, *Fróes 20873* (Be); basin of Rio Negro, *Murça Pires 1121.*

The species has been known hitherto only from the type collection from Venezuela and from a single collection from the basin of Rio Negro in Brazil.

6. ABUTA PANURENSIS Eichl. BRAZIL—Amazonas: basin of Rio Negro, *Murça Pires 446* (Be).

8. ABUTA IMENE (Mart.) Eichl. BRAZIL—Amazonas: basin of Rio Negro, *Ducke 1969; Murça Pires 399* (Vaupez). Pará: basin of Rio Tocantins, *Fróes 23457.*

This is the first record of the species from the basin of Rio Tocantins.

10. ABUTA TRINERVIS (Rusby) Moldenke. BRAZIL—Matto Grosso: Rio Arinos, *J. G. Kuhlmann 250-K.*

11. ABUTA GRANDIFOLIA (Mart.) Sandw. COLOMBIA—Putumayo: basin of Rio Putumayo, *Schultes 3425.* BRAZIL—Amazonas: basin of Rio Solimoes, *Fróes 20865* (Be); basin of Rio Negro, *Fróes 22201, 22111, 20560* (Be); *Murça Pires & Black 1017* (Be); *Murça Pires 507, 1015* (Be); *Schultes & Lopez 9192.* Pará: basin of Rio Tapajos, *Black 47-1801, 47-1009* (Be); basin of Rio Tocantins, *Fróes 23555;* basin of Rio Cuminá, *A. J. de Sampaio 5231* (Ja), *5243* (Ja), *5257* (Ja). Matto Grosso: Cuiaba, *Hoehne 45111* (Ja); basin of Rio Tapajos *J. G. Kuhlmann 248-K* (Ja). Ceará: *Luetzelburg 20886* (Ja), *20923* (Ja).

This is the first record of the species from the State of Ceará.

12. ABUTA KLUGII Moldenke. BRITISH GUIANA—Demerara: *Forest Dept. 3950.* BRAZIL—Amazonas: basin of Rio Solimoes, *Fróes 20977* (Be); basin of Rio Jutahy, *Fróes 21018* (Be); basin of Rio Negro, *Fróes 21142* (Be), *22561* (Padauiry).

The species has been known hitherto only from the type collection from Peru and from a single collection from the basin of Rio Madeira in Brazil.

13. ABUTA SELLOANA Eichl. BRAZIL—Minas Geraes: *Mello Barreto 1777* (Ja); munic. de Tombos, *J. Evangelista de Oliveira s.n.* (May 9, 1941); munic. de Caeté, *Mendes Magalhães 2388* (Be). Rio de Janeiro: *Ule 3964* (Ja); *Sampaio 2318* (Ja); *Neves Armond 159* (Ja); *Mus. Nac. Rio Jan.*

45414 (Ja). Santa Catarina: *Schwacke 107*, collection IV (Ja). State undesignated; *Mus. Nac. Rio Jan. 45411* (Ja); *Löfgren 291* (Ja).

This is the first record of the species from the State of Santa Catarina.

14. ABUTA RUFESCENS Aubl. COLOMBIA—Vaupés: between Cucui and the mouth of Rio Içana, *Schultes & Lopez 9551*. BRAZIL—Amazonas: basin of Rio Negro, *Mello Filho 566* (near Manaos; Ja); *Schultes & Lopez 8948* (Tapurucuara), *9827a* (Rio Curicuriari); *Schultes & Murça Pires 9029, 9098, 9099* (Vaupés). Pará: basin of Rio Tocantins, *Fróes 23499*. Espirito Santo: basin of Rio Doce, between its affluent Rio Pancas and the town of Linhares, *OVB-6, OVB-20, OVB-45, OVB-46, OVB-51, OVB-55, OVB-74*. Rio de Janeiro: *Mus. Nac. Rio Jan. 45400* (Ja), *45413* (*Mendanha s.n.*) (Ja), *25097* (*Schwacke s.n.*) (Ja); *A. J. de Sampaio 8238* (Ja).

This is the first record of the species from Colombia and from the State of Espirito Santo in Brazil.

15. ABUTA BARBATA Miers. BRAZIL—Pará: basin of Rio Tocantins, ? *Fróes 23382*, ? *23451*.

16. ABUTA GRISEBACHII Triana & Planch. PERU—San Martín: Shapaja, *Klug 132*, BRAZIL—Amazonas: Comatian, *Fróes 23483A;* basin of Rio Jutahy, *Fróes 21014;* basin of Rio Negro, *Murça Pires 377* (foz do Vaupés), 489 (Marabitanas); *Fróes 20489* (Manaos), *22130*, (?) *22209, 22370*.

This is the first record of the species from Peru and from the basin of Rio Jutahy (Amazonas, Brazil).

17. ABUTA CANDOLLEI Triana & Planch. BRAZIL—Amazonas: basin of Rio Negro, *Fróes 22859*. Pará: near Belém, *Ducke 2162* (Be).

18. ABUTA SPLENDIDA Krukoff & Moldenke. BRAZIL—Pará: basin of Rio Tocantins. ? *Fróes 23452*.

ELISSARRHENA MIERS

1. ELISSARRHENA GRANDIFOLIA (Eichl.) Diels. PERU—Loreto: Iquitos, *Massey 2* (March 1947) (K). BRAZIL—Amazonas: basin of the upper Rio Jurua, munic. Eirunepe, ? *Fróes 21723;* basin of Rio Negro, *Murça Pires 239;* Comitian, *Fróes 23957*.

The fruits of *Fróes 23957* differ markedly from those on other material previously cited as this species and there is therefore some doubt in our minds whether all this material is really correctly placed here. In the Fróes collection cited above the fruiting-pedicels are 1 or 2 per axil, axillary or slightly supra-axillary, very stout, nigrescent, 2.5–3.5 cm. long, 4–4.5 mm. in diameter from the base to near the apex, expanded to 8–10 mm. at the apex, glabrous; torus club-shaped, 1–1.4 cm. wide, bearing 1–3 fruits and, upon their falling off, 1–3 conspicuous scars; fruit very hard and dry, yellow when fresh (according to Fróes), nigrescent in drying, elliptic-asymmetric, about 5 cm. long and 3 cm. wide, finely puberulent, glabrescent in age; ectocarp coriaceous or bony, and 3 mm. thick; mesocarp woody, yellowish, about 1 mm. thick, easily separating from the ectocarp; endocarp chartaceous, smooth, shiny, stramineous.

NEW YORK BOTANICAL GARDEN
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TORREYA

INTERESTING WEEDS IN NEW YORK CITY

HARRY E. AHLES

The following is an account of plants recently introduced with ballast and rubbish in the southeastern portion of Bronx County, New York. All the areas from which collections have been made lie south of Pelham Parkway and east of Southern Boulevard. The points of collection provide nine distinct areas. Four localities directly on the East River and its tributaries are trash dumps that have been partially covered with ballast. The other five localities are much smaller and consist entirely of ballast which has been used to raise the level of the roads. Plants collected from the latter have almost certainly been introduced with the soil. Those of the former could just as well have been introduced with the trash as with the ballast.

The plants listed are arranged according to locality. All the specimens were collected by the author and are in his herbarium or the herbarium of the University of Illinois. Collection numbers are given directly after the name of each plant and the numbers are followed by the date of collection. If the country of origin is not given it may be assumed to be Europe.

The soil in which the plants grew is quite different from any previously seen by the author around New York. It is gritty and contains a high percentage of chert in the form of pebbles. This soil has obviously been brought in from some other place and used as fill. Inquiry about the neighborhood and official New York offices as to the source of the soil was futile. I felt quite sure, however, that it is ballast, and the specimens were labeled as growing in ballast. The author's attention was first drawn to these plants while collecting in the vicinity of Westchester Square, June 16, 1946. At this time *Carduus nutans* and *Reseda luteola* were observed growing on the embankment of the Hutchinson River Parkway. On May 18, 1948, a second locality consisting of the same type of soil was located at Halsey Street and Zerega Avenue. After further search, seven additional localities were discovered in which these intersting introduced weeds could be collected.

Diplotaxis tenuifolia is the most abundant and widely distributed, being found at all the waterfront localities except the Castle Hill Area. In the inland localities it occurs only at the Bronx Park area.

1. HUTCHINSON RIVER PARKWAY NEAR WESTCHESTER SQUARE. (None of the plants listed for this locality persisted for any great length of time.)

Reseda luteola L. No. 1097, June 16, 1946. Plants not found in 1950. *Lotus corniculatus* subsp. *eucorniculatus* var. *arvensis* forma *parvifolius* Peterm. No. 1007, July 10, 1946. This form of *Lotus corniculatus* is dwarf and compact. The leaves are small and closely set on the stems. Individual plants measure about a foot and a half across, and have the general appearance of *Thymus serpyllum*. The plants persisted for some time, but did not survive the winter of 1949–50. This specimen was determined by E. J. Alexander of the New York Botanical Garden. *Carduus nutans* L. June 16, 1946; No. 695, July 9, 1948. No plants appeared in the summer of 1950.

2. CASTLE HILL AREA. (Lies between Pugsley's and Westchester Creeks south of Randall Avenue.)

Agrostis spica-venti L. No. 664, July 2, 1948. This specimen was determined by J. R. Swallen. *Polygonum tomentosum* Schrank Nos. 661, 662, 663, July 2, 1948. This is an extremely variable species somewhat resembling *P. persicaria*. Some

plants are low and spreading, others are erect. The leaves vary from lanceolate to ovate, with acuminate to obtuse apices. The lower leaves are quite pubescent, while the upper leaves are nearly glabrous. Fairly abundant in this locality, but, much more common in the 177th Street locality. Still remaining abundant in 1950. *Rumex conglomeratus* Murr. No. 793, Aug. 12, 1948. Plants did not persist for more than one year. *Corispermum hyssopifolium* L. No. 796, Aug. 12, 1948. Native of Eurasia. Only a few plants observed. *Berteroa incana* (L.) DC. No. 657, July 2, 1948. This species is still abundant here and is apparently spreading. *Brassica eruca* L. No. 654, July 2, 1948. Not seen after the first collection. *Galega officinalis* L. No. 665, July 2, 1948; 792, Aug. 12, 1948. Native of Eurasia. Not seen in 1949 or 1950. *Linum usitatissimum* L. No. 666, July 2, 1948. Very abundant in 1948, but, not seen in 1949 or 1950. *Anethum graveolens* L. No. 791, Aug. 12, 1948. Native of Asia. Not seen in 1949 or 1950. *Heliotropium europaeum* L. No. 857, July 2, 1948. Very scarce in 1948 and not seen in 1949 or 1950. *Solanum villosum* Lam. No. 794, Aug. 12, 1948. Native of Eurasia. The plants still persisting in 1950. *Matricaria inodora* L. No. 656, July 2, 1948. No plants seen in 1950.

3. Bronx River Parkway Extension. (Near Sound View Station.)

Artemisia annua L. No. 869, Sept. 13, 1948. Native of Eurasia. Very abundant, appearing to have been here before the introduction of ballast soil. *Eupatorium serotinum* Michx. No. 871, Sept. 13, 1948. Native in North America. Appears to have been established here for some time. *Picris hieracioides* L. No. 868, Sept. 13, 1948. Not too abundant and in an isolated colony.

4. 177th Street Area. (Lies east of the Hutchinson River Parkway and west of Tremont Avenue on 177th Street.)

Cyperus alternifolius L. No. 941, Oct. 14, 1948. This plant is a native of Africa and apparently not hardy enough to persist at this location. The plants survived the winter of 1947–48, however. *Polygonum tomentosum* Schrank No. 945, Oct. 14, 1948; Nos. 1237, 1242, 1250, June 13, 1949. Plants showing the same variation as in the Castle Hill area. Plants still persisting and spreading. *Chenopodium glaucum* L. No. 1249, June 13, 1949; No. 2453, June 6, 1950. Very abundant in 1950. Apparently not introduced with ballast soil as it is growing abundantly in a nearby cultivated field. This plant was also found at the Baychester dump, which has had no ballast soil added. *Corispermum hyssopifolium* L. No. 2447, June 13, 1949. Very scarce, not more than a half dozen plants seen. *Amaranthus viridis* L. No. 950, Oct. 14, 1948. Native of South America. This plant was growing with *Digitaria sanguinalis* var. *ciliaris* (Retz.) Parl. *Arenaria leptoclados* Guss. No. 1243, June 13, 1949. Only a few plants observed. *Stellaria aquatica* (L.) Scop. No. 1251, June 13, 1949. Scarce at this locality, but a large colony has persisted for many years in a wet meadow north of the New York Botanical Garden. *Papaver somniferum* L. No. 1224, June 13, 1949. Plants not uncommon in 1949, however none were seen in 1950. *Diplotaxis muralis* (L.) DC. No. 883, Sept. 23, 1948. This plant is spreading rapidly and in 1950 was found in a larger radius than in 1949. *Diplotaxis tenuifolia* (L.) DC. No. 882, Sept. 23, 1948. Still fairly abundant in 1950, but, not near as common as it was in 1948. *Lepidium perfoliatum* L. No. 1234, June 13, 1949. Only one plant found. *Reseda luteola* L. No. 1229, June 13, 1949. Only a few plants found. Not found in 1950. *Melilotus indica* (L.) All. No. 951, Oct. 14, 1948; No. 1277, June 19, 1949. Native of Eurasia. Not seen in 1950. *Trifolium dubium* Sibth. No. 1239, June 13, 1949. Only one plant found. *Carum carvi* L. No. 1285, June 19, 1949. Although this plant has been known to become established in other parts of New York, it did not persist here. No plants could be found in 1950. *Coriandrum sativum* L. No. 1236, June 13, 1949; No. 2448, June 6, 1950. Native of Eurasia. Plants with very showy pink flowers. Plants were more abundant in 1950 than in 1949. *Heliotropium europaeum* L. No. 887, Sept. 23, 1948. Not found in 1950. *Solanum villosum* Lam. No. 884, Sept. 23, 1948; No. 954, Oct. 14, 1948; No. 1284,

June 13, 1949; No. 1686, Sept. 12, 1949. Plants growing with *Solanum nigrum* L. and *Amaranthus spinosus* L. Common in 1950. *Linaria maroccana* Hook. f. No. 1230, June 13, 1949. Native of northern Africa. Very few plants, and none in 1950. *Verbascum lychnitis* forma *alba* (Mill.) House No. 1283, June 19, 1949. All plants observed in this locality and the Sound View area had white flowers. *Plantago media* L. No. 1287, June 19, 1949. Only one plant found. *Acanthospermum humile* DC. No. 955, Oct. 14, 1948. Native of tropical America. Plants found again in 1949, but not in 1950. *Carduus nutans* L. No. 1266, June 19, 1949. Plants not seen in 1950. *Guizotia abyssinica* Cass. No. 952, Oct. 14, 1948. Native of tropical Africa. Plants very scarce. Not seen in 1949 or 1950. *Matricaria inodora* L. No. 1226, June 13, 1949. Plants quite tall and bushy, some measuring three feet high and two and a half feet broad. *Senecio viscosus* L. No. 880, Sept. 23, 1948; No. 1286, June 19, 1949. Abundant with *Senecio vulgaris* L. which it resembles very closely.

5. HALSEY STREET NEAR ZEREGA AVENUE.

Cytisus scoparius (L.) Link No. 1975, May 18, 1948. Three plants have been observed here. They show no signs of multiplying. During the winter of 1949–50, they were frozen quite severely.

6. THROGS NECK AREA. (Along Shore Drive in the vicinity of Pennyfield Avenue.)

Diplotaxis tenuifolia (L.) DC. Oct. 5, 1948. Very abundant here.

7. HUNTS POINT AREA. (Lies south of East Bay Avenue and is bordered by the Bronx and East Rivers.)

Festuca arundinacea Schreb. No. 896, Sept. 28, 1948; No. 1180, June 5, 1949. Very robust grass attaining a height of three to four feet. Abundant and forming solid stands as though naturalized some time ago. *Amaranthus viridis* L. No. 938, Oct. 6, 1948. Rare, *Cakile maritima* Scop. No. 897, Sept. 28, 1948; No. 1170, June 5, 1949. Common with *Cakile americana* Nutt. Still abundant in 1950. *Diplotaxis tenuifolia* (L.) DC. No. 1175, June 6, 1949. Plants very abundant here. The fleshy roots are collected by the Italian people in this neighborhood. *Lotus corniculatus* L. Oct. 6, 1948. This plant is rather abundant in the vicinity of New York, and in many places very well established. At this and the 177th Street and the Bronx Park area it seems to have made its appearance with the ballast soil. *Galega officinalis* L. No. 939, Oct. 6, 1948. About four plants observed in this locality. *Coriandrum sativum* L. No. 1179, June 5, 1949. Very scarce here. *Ballota nigra* L. No. 1172, June 5, 1949. Scarce, growing with *Diplotaxis*. *Artemisia annua* L. No. 898, Sept. 28, 1948. Abundant here and apparently spreading. *Artemisia biennis* Willd. No. 901, Sept. 28, 1948. Native of North America. Still abundant in 1950. *Eupatorium serotinum* Michx. June 1950. Not as abundant here as in the Sound View area. *Guizotia abyssinica* Cass. Oct. 6, 1948. Few plants observed in 1948. It was not seen after 1948. *Matricaria inodora* L. No. 1174, June 9, 1949. Fairly common.

8. SOUND VIEW AREA. (Lies south of Gildersleeve Avenue and is bordered by the Bronx River and Pugsley's Creek.)

Hordeum jubatum L. No. 1264, June 14, 1949. Native of North America. One small colony appearing as though established. This plant also occurs in the vicinity of Bronx Beach, Throgs Neck, New York, at the edge of a salt marsh. *Cakile maritima* Scop. No. 1265, June 14, 1949. Not nearly as abundant here as in the Hunts Point area. *Diplotaxis tenuifolia* (L.) DC. June 14, 1949. Common. *Verbascum lychnitis* forma *alba* (Mill.) House No. 1256, June 14, 1949. *Eupatorium serotinum* Michx. No. 871, Sept. 13, 1948. This southern and midwestern species has become well established along the Bronx River. Hundreds of plants grow in profusion, appearing as though it had become established many years ago.

9. BRONX RIVER PARKWAY EXTENSION AT BRONX PARK.

Atriplex tatarica L. No. 771, Aug. 2, 1948; No. 856, Aug. 29, 1948; No. 890, Sept. 24, 1948. Native of Eurasia. *Diplotaxis tenuifolia* (L.) DC. Aug. 2, 1948.

Verbena officinalis L. No. 860, Sept. 1, 1948. *Eupatorium serotinum* Michx. Aug. 2, 1948. *Inula graveolens* (L.) Desf. No. 891, Sept. 24, 1948. Specimen determined by E. J. Alexander. *Matricaria inodora* L. No. 1174, Sept. 1, 1948.

This last locality is the area covered by Dr. H. N. Moldenke under the title, "Interesting New Records and Range Extensions," in the Bulletin of the Torrey Club 77: 45, 1950.

The forty-six species collected in New York City since 1946 do not represent all possible ballast introductions for numerous common species such as *Melilotus officinalis* and *Medicago lupulina* have been omitted, although they appear to have been introduced here with the soil. In the summer of 1950 about sixty-seven percent of the species was still persisting.

This collection is represented by indigenous species of five continents. Australia is the only one not represented. Europe is represented with thirty species, Africa three, South America two and Asia one. The remaining seven are from Eurasia.

Of the nineteen families represented, Compositae are the largest with ten species, six are in Cruciferae and five are in Leguminosae. The remaining families are represented with three or fewer species.—DEPARTMENT OF BOTANY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS.

Yellows Diseases of Plants. Of all the known yellows diseases and their causal agents, aster yellows and the aster yellows virus are understood best. Insofar as has been determined, yellows diseases are spread by leafhoppers. In order to indicate the part leafhoppers play in distribution of these diseases, a somewhat detailed description of the relationship of the aster leafhopper, *Macrosteles divisus*, to the spread of aster yellows will be given. A number of different leafhoppers feed on aster plants but only one, the gray aster leafhopper, is known to transmit aster yellows. This insect feeds and breeds on many different species and is numerous everywhere. It might be supposed by anyone not familiar with the situation that every susceptible plant growing outside would get aster yellows early in the season. This, however, is not the case. Although the incidence of aster yellows is high in some seasons, many susceptible plants escape every year. There are several reasons why this occurs. Aster yellows does not pass from the mother insect to the egg and the aster leafhopper does not live over winter in this region. Each leafhopper when it is hatched is free of virus and remains so until it accidentally feeds on a diseased plant. Also, every plant that grows from seed is free of aster yellows virus, since the virus is not passed in seeds. Aster yellows virus must overwinter either in some biennial or perennial plant or in a greenhouse in an annual plant that is growing there. The leafhopper may feed on a diseased plant without picking up virus. If it feeds on such a plant for as long as one hour, it sometimes becomes viruliferous but not always. If it feeds for as long as a week on a diseased plant, it is almost but not quite certain to pick up virus. If it feeds for as long as two weeks on a diseased plant, it is sure to become a potential vector.

If a viruliferous aster leafhopper gets into a planting of healthy asters and spends a week or more there it might be supposed that aster yellows virus would be taken to a large number of plants, but such is not always the case. In fact, it might not transmit to a single plant. A period of from nine days to three weeks must elapse between the time when a leafhopper first feeds on a diseased plant and the time when it is first able to transmit virus. During this period, which is known as the incubation period of the virus in the insect, the plants on which it feeds are spared. If weather conditions are favorable the insect is likely to become infective about two weeks after it first feeds on a diseased plant. Only after this incubation period is it able to transmit virus. However, an infective insect does not transmit virus to every plant on which it feeds for even a whole day. Infective leafhoppers usually remain infective as long as they live, and if conditions are favorable a few insects may spread aster yellows far and wide in a short time. But if there is a hot spell with temperatures running up to 100° F. or higher, the insect ceases to be infective. If the period of hot weather lasts only a few days and is followed by daily temperatures not exceeding 80° F., the leafhopper that has lost its ability to transmit will, without again feeding on a diseased plant, regain this ability. However, it will

not regain ability to transmit at once. A period of time, known as the heat-induced incubation period in the insect, must elapse before it can again transmit. This period may be only a few hours or it may be as long as two weeks. An insect that regains ability to transmit, will transmit as efficiently as before the hot spell. But if the hot weather lasts for two weeks as it does sometimes, especially in the middle and southern states, then the leafhopper will lose ability to transmit permanently. If it ever transmits again, it must again feed on a diseased plant and the virus must again undergo an incubation period in the insect's body just as it did before the insect became a vector. Because of the effect of heat on the virus, it spreads slowly or not at all during midsummer. High temperatures inactivate aster yellows virus both in the insect vector and in the plant. The insect, being small, loses virus quickly. The plant, being relatively large and having roots in the earth where temperature changes occur only after a lag period, retains the virus tenaciously. However, it is possible to cure even a large plant by heat treatments.

As autumn approaches and the weather becomes cooler, the rate of spread of aster yellows increases, but by this time many of the leafhoppers will have become old. With age they feed less and move about less than they did during the prime of life. This tends to hold down the autumn rate of spread. By the end of the season many aster plants will be diseased unless they have been grown in a greenhouse, near a building, or have been protected by a spray, a cloth fence, or in some other way. Plants that escape infection bear viable seeds, while those that take the disease produce either no seeds at all or seeds that are not viable. Most diseased plants live much longer than healthy plants. Many live until frost kills them. In nature aster yellows spreads only through the aster leafhopper. It cannot be taken to healthy plants mechanically except through grafting.

We now know that the virus of aster yellows multiplies both in the insect and in the plant. We believe that the incubation period and the heat-induced incubation period in the insect are the periods during which the virus multiplies in cells of the leafhopper and is released in sufficient quantity to contaminate the saliva. In nature the virus multiplies alternately in insect and in plant. Like the plant rusts, it is heteroecious and, like rusts, it cannot be cultivated in artificial media. Multiplication of aster yellows virus in its insect host causes no detectable symptoms of disease. Multiplication in its plant host causes a variety of symptoms,—chlorosis, abnormal branching, upright habit of growth, stunting, malformations, virescence of flowers, sterility, and increased length of life. The latter effect might be considered advantageous to the plant, but of what advantage is longevity when beauty of flowers and viability of seeds are destroyed.

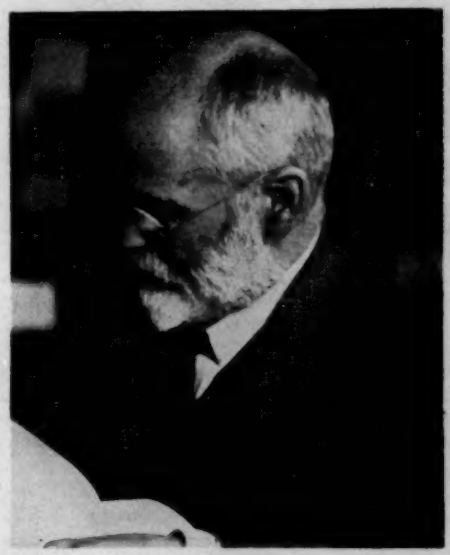
Aster yellows virus has not yet been identified under the electron microscope. It has not been crystallized. It has been transmitted mechanically from leafhopper to leafhopper and from plant to leafhopper, but not from leafhopper to plant or from plant to plant. It has been transmitted to species belonging in more than forty different families of plants by means of the leafhopper vector. Susceptible species are found in both the Monocotyledoneae and the Dicotyledoneae. Some genera have species that are highly susceptible and species that are immune. Many different plants are naturally immune. Some, to which the virus either cannot be transmitted by means of the aster leafhopper or is transmitted with great difficulty by this insect, are infected by grafting or by means of dodder. The great advantage of using dodder in transmission of aster yellows results from the ability of this parasite to pass virus between plants so distantly related that they do not unite when grafted together, and from its abiliy to take virus to plants that are poisonous or unpalatable to the insect vector.—L. O. KUNKEL, Rockefeller Institute for Medical Research.

MERRITT LYNDON FERNALD. 1873–1950*

Merritt Lyndon Fernald. On September 22, 1950, Merritt Lyndon Fernald, one of America's most widely known and respected botanists, died at his home in Cambridge, Massachusetts. The son of Dr. Merritt Caldwell Fernald, first and third president of Maine State College of Agriculture and Mechanic Arts (now the University of Maine), he was

* Photo by E. C. Abbe, courtesy of Gray Herbarium.

born October 5, 1873, at Orono, where his early training was received. After completing high school, he had entered the State College as a freshman in 1890 when Sereno Watson, then Curator of the Gray Herbarium, invited him to become an assistant there. Fernald accepted the assistantship at the Gray Herbarium early in 1891 and in the fall registered as a student in Harvard's Lawrence Scientific School, from which he received the degree of Bachelor of Science, *magna cum laude*, in 1897. It is a remarkable fact that his intimate association with the Gray Herbarium, of which he was Curator from 1935 to 1937 and Director from 1937 to 1947, extended over a period of nearly sixty years to the time of his death. He was successively Instructor, Assistant Professor, and Fisher Professor of Natural History in Harvard University, becoming Emeritus in 1947.



Fernald's botanical career began even before he entered college and might be appropriately calculated from his first paper, published in 1890. This was a short note concerning two taxa in *Carex* which appeared in the BULLETIN OF THE TORREY BOTANICAL CLUB. It was followed by a second article in the same journal the next year. These first papers grew out of field studies involving critical observations on the plants growing in their native habitats. From this time forward, except for a short interlude in which he studied Mexican and subtropical groups, he combined field and herbarium activities in the elucidation of the problems he undertook to solve. Through his many botanical expeditions, at first in Maine and later in Quebec, Newfoundland, Michigan, and Virginia, together with numerous shorter trips from Cambridge, he gained a first-hand knowledge of the plants of eastern North America that was truly remarkable. He could talk for hours, without notes, concerning details of species differences, habitat preferences and geographical ranges of the plants of a vast area stretching from Virginia to Newfoundland, westward to the Mississippi. For a long period he has been the unchallenged authority on the flora of this area.

Throughout his life, Fernald was vigorous in his research which resulted in more than 750 published papers. He collaborated with B. L. Robinson to produce the seventh edition of Gray's Manual, published in 1908, and became the sole author[1] of the eighth

[1] He was ably assisted, at the critical stages of manuscript preparation, organization, and proofwork, by Dr. Bernice G. Schubert.

edition which came from the press only a few months before his death. The rewriting and expansion of this century-old classic had occupied a large share of his time for nearly fifteen years. Though his health was good, he found the tasks involved in the final preparation and printing of this 1600-page book quite taxing, and it is more than probable that his extraordinary exertions in this connection were a contributing factor toward his death. Certainly this monumental and authoritative work on the flora of eastern North America stands as a crowning achievement to a long and effective botanical career.

Fernald was many-sided in his botanical activities. The subject matter of the larger portion of his published papers would undoubtedly be classed as floristic, but in many of these he included partial revisions of difficult groups and often tangled with knotty problems in nomenclature. For many years such revisionary work was focused on the prospective "eighth edition", but on occasion, as with *Potomogeton*,[2] it grew into a full-scale monograph. Similarly, *Draba* produced a challenge that stimulated him to monograph the genus for eastern North America.

Acute observations on the ranges of plants ultimately led to phytogeographic studies of far-reaching importance. He was early impressed with cases where species commonly found in the Rocky Mountains or the Cascades of western America were isolated in small unglaciated spots in Newfoundland or on the Gaspé Peninsula, etc. In attempting to explain these in terms of the history of the areas in which they occurred, he ran headlong into the accepted dogma that the plant cover had been completely destroyed by the ice sheets of the glacial period. He argued tenaciously and presented convincing evidence that certain sites were not glaciated and that they had remained habitable for those now isolated plants that have persisted to the present. His studies along this line dealt not only with the boreal elements of the flora but with the southern components and those primarily of other continents. His essays on plant geography were logical, clearly presented and backed with critically gathered data. The conclusions arrived at have not always been fully concurred in by geologists and others but the results and conclusions of his studies cannot be ignored. They have served as a powerful stimulus toward a critical reexamination of the evidence upon which the nature and extent of the ice of the glacial age have been determined.

Professor Fernald was intolerant of careless work and often sharply criticized other botanists in his lectures and writings. His role as a critic was heightened by the fact that he handled so many manuscripts and books for review in connection with his editorship of RHODORA. He was a member of the first editorial board, beginning in 1899, and became its editor-in-chief in 1928. One of Fernald's outstanding traits was his abundant enthusiasm for botany. This could be touched off by items of small or large significance and was one of the most important factors that kept him working at high speed throughout his entire life. The days and years were spent absorbed in the work that he loved and his preeminence in the science of botany attests to the quality of the publications resulting therefrom.—R. C. ROLLINS, The Gray Herbarium, Harvard University.

BOOK REVIEW

An Introduction to the Embryology of Angiosperms. P. Maheshwari, Professor of Botany, University of Delhi, Delhi, India. X + 453 pages. Fig. 1–216. McGraw-Hill Book Co., Inc., New York, Toronto, London. 1950. $6.00.

This book is an excellent resumé to date on the origin and development of the angiosperm embryo. As such it is the first comprehensive piece of work on any phase of angiosperm development, written in English, since the publication of Chamberlain's "Morphology of Angiosperms" in 1903. The author has compiled his information from papers in the various countries, east and west, where botanical research is being carried on.

This book meets a timely need. We who teach a course in the comparative morphology of the higher plants often find ourselves wishing that a textbook(s) that does for the

[2] The Linear-Leaved North American Species of Potomogeton, Section Axillares. Mem. Amer. Acad. Arts & Sciences 27: 1–183, 1932.

Angiosperms what has already been done for the lower plant groups, were available for student use. This is a good beginning.

Embryology, of course, means the study of the development of the embryo, but in plants this development is so intimately tied in with the origin and development of the gametophyte, that is, with the events which lead up to the formation of the embryo and its accompanying endosperm, that one would be incomplete without the other. The author, therefore, includes both the gametophyte and the embryo, and while he does justice to the former, the feeling of this reviewer is that the development of the embryo in a group as large as is that of the angiosperms warrants fuller treatment. Embryos are classified under five general types plus the "unclassified or abnormal embryos" and "the unorganized and reduced embryos." Little or no mention is made of embryo development in such large and important families as the Leguminosae, Rosaceae (two mentioned) and the primitive family Ranunculaceae (one mentioned).

What would seem, to this reviewer, to be a pretty complete bibliography accompanies each chapter. Footnotes are occasionally used to further supplement reference to the particular phase under discussion. The numerous illustrations throughout the book add considerably to its clarity. In fact, without these drawings there are times when the reader will be at a loss to understand the meaning of unfamiliar terms. According to the author he has borrowed these illustrations freely from the works of others, very few are original.

The book opens with a brief historical sketch of sexuality in plants as a background for the discussion of the origin and development of the male and female gametophytes from the microsporangium and the megasporangium, respectively, with the variations found accompanying each. Typical and variational types are well documented with examples in all cases. A chapter on fertilization is followed by one each on embryo and endosperm development. Apomixis and polyembryony each rate a chapter.

Formerly disputed points are being cleared up gradually as research progresses; for example, cell division in tapetal cells is found to be mitotic, not amitotic as formerly believed; division of the generative cell, whether this occurs in the pollen grain or in the cytoplasm of the pollen tube is followed by the formation of sperm *cells*, that is, "in all cases the male gametes are definite cells." The term megasporangium is used as synonymous with ovule.

Embryology has three disciplines: descriptive (subjective matter of this text), phylogenetic and experimental. The author is hopeful that the first can be of service to the latter two in solving some of their more difficult and obscure problems. For example, he cites eleven possible sources of aid; nature of the anther tapetum, megasporogenesis, development of the embryo sac, etc., where descriptive embryology may help throw some light on relationships of doubtful groups.

A summary of the present position of experimental embryology is given under the following topics: control of fertilization; embryo culture; induced parthenogenesis; production of adventive embryos; and induced parthenocarpy. One working in this field of botany where the geneticist, physiologist, cytologist and embryologist can meet on common ground, will find something of interest to him. Pollination and fertilization may be effective but the embryo fails to develop. Why?

The final chapter, "Theoretical Conclusions," sums up some of the current theories of possible angiosperm origin, long "a thorn in the flesh" to all botanists. After pretty thoroughly combing the gymnosperms as the best possible ancestral source the author concludes that while it may be possible to derive the male gametophyte of the angiosperm from that of some gymnospermous ancestor the same cannot be said so readily of the female gametophyte. That the angiosperms are probably a monophyletic group is supported by the facts—(1) that there are no essential differences within the group "as regards the development and organization of the male and female gametophytes and the endosperm", and (2) the process of double fertilization. The differences in the organization of the embryo between monocotyledons and dicotyledons "are not fundamental," some dicotyledons have one fully developed cotyledon while some monocotyledons are known in which both cotyledons are well-developed.

For further enlightenment regarding angiosperm development and relationships

botanists are now centering their hopes on some of the less well known members of the Degeneriaceae, Winteraceae, etc.—HETTIE M. CHUTE, New Jersey College for Women, Rutgers University, The State University of New Jersey.

PROCEEDINGS OF THE CLUB

Minutes of the Meeting of November 15, 1949. The meeting, at Hunter College, was opened at 8: 10 P.M. by President Matzke; 43 members and friends were present. The minutes of the preceding meeting were read and approved. Dr. Wanda K. Farr of the Celanese Corporation spoke on "Crystalline and Amorphous Cellulose".

After much discussion the meeting was adjourned at 9: 30 P.M. Refreshments were served by the Hunter College botanists.

Minutes of the Meeting of April 19, 1950. After refreshments, served by members of Fordham University, the meeting was called to order at Fordham at 4: 10 P.M.; 34 members and friends were present. Dr. C. A. Berger addressed the Club on "Induced vascular differentiation". [Father Berger's paper is to be published in the Bulletin.]

After a good deal of discussion the meeting was adjourned at 5: 00 P.M.

Minutes of the Meeting of October 17, 1950. The meeting, at the New York Academy of Sciences, was called to order at 8: 10 P.M. by President Clum. Reports were given on the VII International Botanical Congress at Stockholm. D. P. Rogers discussed chiefly the nomenclatorial sessions and decisions. Dr. Lela V. Barton gave an account of her explorations and discoveries in Stockholm and Upsala, illustrating her points with Kodachromes. Dr. Clyde Chandler showed a series of Kodachromes illuminating her account of the Kew gardens and of a number of Swedish experiment stations. Refreshments were served after the meeting.

Respectfully submitted,
DONALD P. ROGERS,
Recording Secretary

Minutes of the Meeting of October 31, 1950. The meeting was called to order at 8: 00 P.M. at the New York Academy of Sciences Building by the President, Dr. Clum. There were twenty-six members and friends present. The reading of the minutes of the preceding meetings was postponed. Dr. Lindsay S. Olive of the Botany Department of Columbia University then presented "A Review of Recent Investigations on Genetics of Glomerella." The talk was illustrated with lantern slides. The speaker's abstract follows:

> The recent papers of Edgerton and his co-workers on the genetics and cytology of *Glomerella* were reviewed. Two types of "plus" strains, two "minus" strains, and two different conidial strains were studied genetically. The inheritance of two pairs of allelomorphic factors has been studied intensively. Gene *A* controls the production of perithecia, while its allelomorph *a* controls the production of conidia; *B* controls the grouping of perithecia or conidia in large masses, while the presence of the allelomorph *b* results in a scattered arrangement of perithecia or conidial masses. Therefore, "plus" cultures, which were found to possess the genotype AB, produce perithecia in large glomerate masses, while "minus" cultures, genotype Ab, have their perithecia scattered singly or in groups of 2 or 3. Conidial A cultures, genotype aB, produce conidia in large masses, but lack perithecia; Conidial B cultures, genotype ab, produce conidia in small scattered groups and also lack perithecia. The Plus A or wild-type strain has been found to differ from Plus B in possessing a mutator gene *M* which appears to influence the mutation of *B* to *b*.

This explains the observation by several investigators that wild-type cultures always give rise to a large number of perithecia whose asci segregate for both "plus" and "minus" cultural types. Although "plus" and "minus" strains, when grown alone, produce perithecia readily (infertile only in Minus A), lines of fertile perithecia will develop between any two paired strains as long as both *A* and *B* factors are represented in the cross. In all such cases cross-fertilization has been demonstrated genetically. Cytological investigations have shown that the perithecia (in single strains or in crosses) have their origin in a pair of uninucleate initials which arise from adjacent cells of a hypha. One initial surrounds the other, the inner one becoming the ascogonial coil, the other developing into the perithecial wall. At an early stage a copulation hypha grows into the young perithecium and fuses with the tip of the ascogonium, thus initiating the dikaryotic phase. In crosses, the copulation hypha has been found to be produced by one strain, the initials by the other. McGahen J. W., and H. E. Wheeler, Genetics of Glomerella. IX. Perithecial development and plasmogamy. In Press.) Croziers proliferate from a single dikaryotic cell of the ascogonial coil. Nuclear fusion occurs in the young asci, and this is followed later by 3 successive nuclear divisions, the first two meiotic, resulting in the production of 8 haploid nuclei. The fusion nucleus contains 4 bivalent chromosomes and each of the 8 haploid nuclei contains 4 univalents. Eight uninucleate ascospores are cut out in the ascus.

Following the question period the meeting was adjourned and refreshments were served.

Respectfully submitted,
HONOR M. HOLLINGHURST
Secretary protem

Minutes of the Meeting of November 8, 1950. The meeting was called to order at Hunter College by President Clum; seventeen members and friends were present. The following were elected to membership in the Club: Active members: Walter V. Brown, Austin, Texas; Henry V. Kivela, Jr., Greendale, Wisconsin; Sidney A. Simmons, Springfield, Massachusetts; Associate members: Fritz G. Hochwald, New York City; Stuart A. Rice, New York City; Olga P. Schwarzbach, Douglaston, New York; L. Colette Siess, Hackensack, New Jersey.

A number of members reported on their summer botanical experiences. Dr. Murray Buell described: (1) observations on prescribed burning in the Pine Barrens: sedges had replaced shrubs in areas where shrub-tree associations had been burnt over; (2) six weeks spent at the University of Minnesota Biological Station; (3) the ecologists' field trip to southern Ohio. Dr. Buell showed kodachrome slides to illustrate his notes and was followed by Dr. Small who discussed further observations made on the southern Ohio trip, illustrated by kodachromes.

Dr. E. H. Fulling showed an excellent series of kodachrome slides taken in and around Stockholm during the Botanical Congress last summer, giving much local color as well as points of botanical interest.

Dr. Lindsay Black gave detailed account of the meeting in Stockholm at which the Russian delegation was present to give four papers presenting the Russian position on genetics. Results based on ratios obtained with higher plants were criticized, because statistical. Results obtained in *Neurospora* were unexplained by the Russians, though the question was asked.

Dr. Shull gave the result of his experiment with 1000 acorns from his *Quercus macrocarpa* tree. Of 67 which germinated, 55 produced green seedlings and 12 albinos.

Dr. Elva Lawton spoke of ferns and mosses collected while she spent the summer at the Iowa Lakeside Laboratory.

President Clum and Dr. Annette Hervey gave their impressions of the botanical meetings at Columbus in September.

Dr. M. A. Johnson gave some observations of his two weeks in Florida, especially of the wide range of *Magnolia virginiana* and its various associates.

Dr. A. E. Hitchcock described the present surroundings and activities of a successfully retired botanist, an ideal picture with a moral.

After refreshments, the meeting adjourned about 10: 00 P.M.

Respectfully submitted,
JENNIE L. S. SIMPSON
Corresponding Secretary

Minutes of the Meeting of December 5, 1950. The meeting was called to order at Hunter College by President Clum at 8: 35 P.M.; 35 members and friends were present. The minutes of the preceding meeting were read and approved.

Henry F. Dunbar, Kingston, N. Y.; Estill I. Green, Short Hills, N. J.; Martin B. Harrison, Manhattan, Kans.; Matilde Weingartner, Staten Island, N. Y.; Agnes White, Richmond Hill, N. Y.; and Warren A. Wistendahl, New Brunswick, N. J., were elected to active membership and Margaret T. Hills, New York City, and Mrs. Albert Blumgart, Newark, N. J., to associate membership.

Dr. Edwin T. Moul of Rutgers University spoke to the Club on "Mosses of Pennsylvania". His abstract follows:

> A study of the moss flora of central and eastern Pennsylvania was undertaken to determine the range of species in the state and the factors influencing their distribution.
>
> The area covered in this study comprises 40 of the 67 counties of the state. The work is based on collections made by the writer and other collectors together with material in the herbaria of the University of Pennsylvania, Academy of Natural Sciences of Philadelphia, New York Botanical Garden, and Chicago Museum of Natural History.
>
> After carefully mapping and compiling data for each species, the following conclusions concerning the distribution of species have been drawn:
>
> The distributional maps of those species of mosses adequately collected showed no correlation between physiographic provinces of the state and their distribution. The amount of precipitation is adequate and evenly distributed throughout the year, and so moisture is not a limiting factor. The northern and central counties of the state are within the "cool" area of Livingston and Shreve, while those in the south-west and south-east corners are within the "medium" temperature area. The natural vegetation of this "cool" area is primarily the Eastern Hemlock forest of Nichols, while that of the "medium" area is the Eastern Deciduous forest, now greatly decimated by agriculture and industrial developments. The greatest abundance of mosses and liverworts, both species and numbers, occurs in the Eastern Hemlock Forest.
>
> Mosses were found to be more responsive to the microclimate than the macroclimate. The interaction of light, temperature, and moisture at the specific habitats of each species controls the microclimate. It is natural therefore to expect to find each species growing wherever local conditions are favorable, thus overstepping the boundaries of physiographic provinces.

Many mosses are confined to specific substrates and form recognizable communities. Most species showed preference for either an acid or alkaline situation. Communities on slopes showed a succession from xeric, acid soil species at the top to mesic, neutral or alkaline soil species at the bottom. Limestone ridges, serpentine and shale barrens showed moss communities peculiar to them.

Four geographic relationships are represented in the moss flora of Pennsylvania. First, a group of mosses with arctic or circumpolar affinities, which reach their southern limits in the state and occur in the northern counties, back of the glacial moraine. Secondly, the mosses showing relationship with those of the coniferous forest and entering Pennsylvania all along the northern boundary, funnelling down the mountain chain through central Pennsylvania to the southern boundary and on into the southern Appalachians. These species are associated with the Eastern Hemlock forest. The third group of mosses are widespread and occur in every suitable habitat in most of the counties of the state. They are characteristic of the Eastern Deciduous forest. The fourth and smallest element is characteristic of the coastal plain. These species are separable into groups. Those of the first group show relationship with species of the southern Appalachians and occur in coastal plain of Pennsylvania, and from there have ascended the Susquehanna and Delaware Rivers and their tributaries into the Mountain and Plateau Provinces. The second group, with tropical relationships, has entered the state in the counties of the southeastern corner and in Fayette county in the southwestern corner. This type of distribution is similar to that of a number of phanerogams found on the coastal plain, which extend up the Mississippi Embayment into western Pennsylvania.

In the present study 249 species of mosses with 32 varieties are reported. Rare species collected during the last century have not been relocated. One of the costs of advancing civilization is the destruction of the forests and the disappearance of these rare species.

After adjournment at 9: 35, refreshments were served by the Hunter College botanists.

Minutes of the Meeting of December 19, 1950. The meeting was called to order at Columbia University at 8:02 P.M. by President Clum; 47 were present. The minutes of the preceding meeting were read and approved.

The proposed budget for 1951 was presented by Dr. A. E. Hitchcock for the Committee which included also Dr. L. M. Black and Dr. H. W. Rickett (chairman). The estimated income for 1951 was $7,450 and the proposed expenditures $8,095. Of the expenditures $6,000 was to be for publication of the BULLETIN; the deficit of $625 was chiefly the result of increased costs of printing. The adoption of this budget was recommended only with the understanding that action would be taken by the Council "to avoid the deficit." A committee was announced by the President to consider means of increasing the Club's income: Dr. Frederick Kavanagh (chairman), Dr. Frank Lier, Dr. Murray Buell. It was then voted without dissent that the budget be adopted.

Dr. L. O. Kunkel of the Rockefeller Institute for Medical Research then addressed the Club on "Yellows Diseases of Plants."

Slides were exhibited showing various yellows diseases on their hosts and on infected tomatoes or carrots, and plants cured by heat.

The meeting was adjourned at 9: 10.

Respectfully submitted,
DONALD P. ROGERS,
Recording Secretary

NOTE

Lilium philadelphicum L. grows in generous abundance, beautifully and normally in Montague Township, Sussex County, N. J. In August 1950 Mr. William Mitchell noticed one plant that appeared to deviate from the normal in shape, as it was actually "square". It was so markedly so that he brought it to me and it was found to have eight petals, eight stamens and a 4-lobed stigma.

With all the living specimens of this species which I have seen growing in Montague, and probably dozens of herbarium specimens in the Local Herbarium of the N. Y. Botanical Garden, during the years I cared for that Herbarium, I have never seen a *Lilium philadelphicum* stray from the straight and narrow path as this one does.

Possibly some other member of the Torrey Botanical Club might supply additional information. I have at present the dried specimen and a water-color sketch.—PALMYRE DE C. MITCHELL, Yonkers, N. Y.

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COMPILED BY

LAZELLA SCHWARTEN

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